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TREE SPECIES EFFECTS ON CD AND ZN MOBILITY AFTER AFFORESTATION OF CONTAMINATED SOILS IN THE CAMPINE REGION (NORTHERN BELGIUM)

Thesis submitted in fulfillment of the requirements
for the degree of Doctor (PhD) in Applied Biological Sciences:
Forest and Nature Management

Dutch translation of the title:
Effecten van boomsoortenkeuze op de Cd- en Zn-mobiliteit bij bebossing van verontreinigde gronden in de Kempen (Noord-België)

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Woord vooraf

Ik moet een jaar of zes geweest zijn. Een kinderboek had me verteld dat eksters verwoede verzamelaars zijn van alles wat schittert en blinkt - hun nesten zouden vol 'gepikte' juwelen liggen. Veel meer glitter en glamour had mijn kinderlijke nieuwsgierigheid niet nodig. Bij gebrek aan juwelen, ontvreemdde ik wat zilverpapier uit de keukenkast, rolde er bolletjes van en legde die zorgvuldig in ons klein stadstuintje. Geen ekster die daaraan zou kunnen weerstaan! Mijn eerste wetenschappelijk experiment was een feit, de proefopzet leek me waterdicht. De daaropvolgende dagen groeide echter mijn teleurstelling want de zilveren bolletjes bleven liggen in het gras. Tot op de dag van vandaag weet ik dus nog steeds niet hoe het interieur van een eksternest eruit ziet…

Mijn eerste kennismaking met 'de exacte wetenschap' mag dan wel teleurstellend afgelopen zijn, mijn fascinatie voor alles wat groeit, bloeit, vliegt, fladdert en kruipt werd er niet minder op. Van jongs af aan wist ik dat ik bioloog wou worden; ik schreef er zelfs een gedicht over in het zesde leerjaar. Maar wat ik niet wist, was dat mijn liefde voor de natuur ooit zou resulteren in dit doctoraat. Bomen dienden toch om in te klimmen?

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Lotte

Contents

Summary

This thesis was executed in the context of the historical metal pollution in the Campine (Kempen) region in north-eastern Belgium. Due to metal refining activities in the past, an extended area of about 700 km² is diffusely polluted with mainly cadmium (Cd) and zinc (Zn). The historical soil pollution is still causing human-toxicological and ecotoxicological risks, through metal leaching to groundwater and accumulation in the food chain. Moreover, the Campine region is characterized by poor sandy soils, aggravating the risks for metal dispersion in the environment.

Conventional soil sanitation techniques are technically and financially inadequate to tackle the pollution problem, because of its spatial extent and the relatively moderate contamination levels. Hence, alternative remediation strategies, such as phytostabilization, are to be investigated.

Sustainable phytostabilization projects require the metals to be stabilized in the soil and should thus aim at minimizing metal dispersion via both above- and below-ground pathways. As there is a clear tree species effect on metal uptake, on litter decomposition, on biogeochemical processes in the soil profile and on the water balance, the distribution and fluxes of metals in the forest-soil system will be tree species specific as well. Selecting appropriate tree species is thus crucial for achieving successful phytostabilization.

The main objectives of this thesis were to assess the feasibility of phytostabilization by afforesting diffusely Cd and Zn contaminated sandy soils, and to determine the tree species effects on Cd and Zn cycling on these soils. Our study was carried out in 'Waaltjesbos', a young post-agricultural forest on a metal contaminated site on sandy soil. At the moment of our study, the trees were 10-15 years old. The six selected tree species were silver birch (*Betula pendula*), oak (*Quercus robur* and *Q. petraea*), black locust (*Robinia pseudoacacia*), aspen (*Populus tremula*), Scots pine (*Pinus sylvestris*) and Douglas fir (*Pseudotsuga menziesii*).

We showed that aspen translocates high amounts of Cd and Zn into its foliage, generating a significant accumulation of total Cd and Zn concentrations in the topsoil (0-5 cm), and this after only 10 years of tree growth. Moreover, accumulated metals in the leaves may pose a long-term

risk to primary consumers and enter the food web. Given these risks of above-ground metal dispersion, aspen should be avoided when afforesting Cd and Zn contaminated sites.

We also showed that Cd and Zn leaching at 50 cm depth was elevated under black locust, Scots pine and Douglas fir, compared to lower seepage fluxes under silver birch, oak and aspen. This pattern was significantly correlated with leaching of anions and base cations, and to a lesser extent to soil solution pH, at least at this young stage of forest development at a post-agricultural site. Due to former (agricultural) liming and fertilization processes, the base cation status of the soil and the solution pH were still relatively high. However, we expect both parameters to decrease with forest age, especially for species with slowly decomposing litter, implying that the effect of pH on Cd and Zn leaching will become relatively more important in the long term. In this respect, we recommend not to plant tree species that generate high anion, base cation or H^+ fluxes when afforesting Cd and Zn contaminated lands, because these species pose a serious risk for metal leaching to deeper soil layers and potential contamination of the groundwater. In general, coniferous as well as N-fixing species should thus be avoided.
Finally, contrary to what is often argued in literature about phytostabilization, we found that Cd and Zn leaching losses at our study site showed no resemblance with the downward soil water fluxes. This might imply that the impact of the biogeochemical processes in the soil (solution) on Cd and Zn leaching (complexation with anions, competition for sorption sites with base cations and protons) was relatively more important than the effect of evapotranspiration.

Summarized, taking into account all the aforementioned recommendations, it seemed that silver birch and oak may be planted on Cd and Zn contaminated sandy soils. Aspen, black locust, Scots pine and Douglas fir, on the other hand, cause risks for above-ground or below-ground metal dispersion and should therefore be avoided.
These conclusions were drawn from our study in a young forest (10 – 15 years). Consequently, it is unsure whether they can be extended to the long term. Further research in older forests is thus essential.

Samenvatting

Dit doctoraat is het sluitstuk van een onderzoek dat werd uitgevoerd in het licht van de problematiek van metaalverontreiniging in de Belgische Kempen. Als gevolg van hoge metaalemissies van de non-ferro industrie uit de vorige eeuw worden we vandaag geconfronteerd met de erfenis van een historische bodemverontreiniging, die zich over een grote oppervlakte in de regio heeft verspreid. Ongeveer 700 km² is diffuus verontreinigd met voornamelijk cadmium (Cd) en zink (Zn). Deze metaalaccumulatie in de bodem zorgt voor risico's voor het ecosyteem en voor de volksgezondheid, onder de vorm van uitloging naar grond- of oppervlaktewater en verspreiding in de voedselketen. De regio wordt bovendien gekenmerkt door arme zandbodems die het risico op uitloging en verspreiding van de metalen in het ecosysteem nog verhogen. Door de uitgestrektheid van de verontreiniging is klassieke bodemsanering geen haalbare kaart. Bijgevolg dringen alternatieve beheertechnieken, zoals fytostabilisatie, zich op.

Duurzame fytostabilisatieprojecten beogen het vastleggen van metalen in de bodem door middel van plantengroei, en dienen er dus voor te zorgen dat zowel boven- als ondergrondse metaalverspreiding geminimaliseerd wordt. Aangezien er duidelijke boomsoorteffecten zijn op metaalopname, strooiselafbraak, biogeochemische processen in het bodemprofiel en op de waterbalans, zal de metaal(re)distributie in het bosecosysteem ook boomsoortspecifiek zijn. Het selecteren van geschikte boomsoorten is dan ook cruciaal om succesvolle fytostabilisatie te realiseren.

De hoofddoelstellingen van dit doctoraat waren om de haalbaarheid te onderzoeken van fytostabilisatie door bebossing van Cd- en Zn-verontreinigde zandbodems, en om de boomsoorteffecten op de Cd- en Zn-fluxen te bepalen. De studie werd uitgevoerd in 'Waaltjesbos', een bos dat ongeveer 15 jaar geleden aangeplant werd op verontreinigde landbouwgrond in Lommel. De zes geselecteerde boomsoorten waren ruwe berk (*Betula pendula*), inlandse eik (*Quercus robur* en *Q. petraea*), valse acacia (*Robinia pseudoacacia*), trilpopulier (*Populus tremula*), grove den (*Pinus sylvestris*) en Douglasspar (*Pseudotsuga menziesii*).

In dit onderzoek toonden we aan dat trilpopulier grote hoeveelheden Cd en Zn naar zijn bladeren transloceert, wat resulteert in een aanzienlijke Cd- en Zn-accumulatie in de bovenste bodemlaag (0-5 cm) - en dit al na slechts 10 jaar. Geaccumuleerde metalen in de bladeren kunnen bovendien een mogelijk risico inhouden voor herbivoren en zo in het voedselweb terechtkomen. Rekening houdend met deze risico's op bovengrondse metaalverspreiding, dient trilpopulier te worden vermeden bij het bebossen van Cd- en Zn-verontreinigde sites.

We toonden ook aan dat de uitspoeling van Cd en Zn op 50 cm diepte aanzienlijk hoger was onder valse acacia, grove den en Douglasspar dan onder ruwe berk, eik en trilpopulier. De uitspoeling van Cd en Zn was duidelijk gecorreleerd aan de uitspoeling van anionen en basische kationen, maar slechts in mindere mate gelinkt aan de pH van de bodemoplossing. Dit laatste was hoogstwaarschijnlijk te wijten aan het jonge stadium van bosontwikkeling op voormalige landbouwgrond. Onder invloed van de voormalige bekalkings- en bemestingspraktijken, is het gehalte aan basische kationen in de bodem en de pH van de bodemoplossing immers nog relatief hoog in onze studiesite. We verwachten echter dat beide parameters zullen dalen naarmate het bos verder ontwikkelt, voornamelijk onder de boomsoorten met trage strooiselafbraak. Dit betekent dat het pH-effect op de uitspoeling van Cd en Zn waarschijnlijk prominenter zal worden op lange termijn. Daarom adviseren we om geen boomsoorten aan te planten die hoge uitspoelingsfluxen van anionen, basische kationen of protonen genereren, aangezien deze soorten een groter risico inhouden op metaaluitloging naar diepere bodemlagen en bijgevolg ook op een potentiële vervuiling van het grondwater. In het algemeen dienen dus zowel naaldbomen als stikstoffixerende boomsoorten vermeden te worden op Cd- en Zn-verontreinigde zandgronden.

Tot slot stelden we vast dat – in tegenstelling tot wat vaak in literatuur over fytostabilisatie gesteld wordt – het uitspoelingspatroon van Cd en Zn onder de verschillende boomsoorten op onze studiesite niet overeen kwam met het patroon van de neerwaartse bodemwaterfluxen. Dit impliceert mogelijks dat de impact van de biogeochemische processen in de bodem(oplossing) op de Cd- en Zn-uitspoeling (complexatie met anionen, competitie voor sorptiesites met basische kationen en protonen) relatief belangrijker was dan het effect van evapotranspiratie.

Samengevat en rekening houdend met alle hierbovengenoemde aanbevelingen, kunnen we besluiten dat ruwe berk en eik de voorkeur genieten wanneer Cd- en Zn-verontreinigde zandgronden bebost worden. Trilpopulier, valse acacia, grove den en Douglasspar daarentegen

veroorzaken risico's voor boven- en ondergrondse metaalverspreiding en dienen daarom vermeden te worden.

Deze conclusies zijn gebaseerd op de resultaten van ons onderzoek in een jong bos (10 – 15 jaar). Bijgevolg is het niet zeker of deze kunnen geïnterpreteerd worden op lange termijn. Verder onderzoek in oudere bossen is dus noodzakelijk.

List of abbreviations and symbols

BC	base cations
CEC	cation exchange capacity
CEC_e	effective cation exchange capacity
diss	dissolved
DOC	dissolved organic carbon
FF	forest floor
I_c	canopy interception loss
ICP-AES	inductively coupled plasma atomic emission spectroscopy
ICP-MS	inductively coupled plasma mass spectrometry
k	litter decomposition rate
K_{sat}	saturated hydraulic conductivity
LAI	leaf area index
LF	litterfall
M_{LF}	annual leaf litterfall
M_0	initial litter mass
M_t	litter mass at time t
n	sample size
OC	organic carbon
Of	fragmentation horizon
Oh	humus horizon
Ol	litter horizon
p	significance of statistical test (p-value)
P	precipitation
pH	measure of acidity
R^2	coefficient of determination
SLA	specific leaf area
spp.	species
st.dev	standard deviation
SWC	volumetric soil water content
θ_r	residual water content
θ_s	saturated water content
T	transpiration
TDR	Time Domain Reflectometry
TF	throughfall
tot	total

1

Introduction

1.1 Historical metal pollution

Industrialization and the lack of effective environmental protection during the first half of the 20th century gave rise to significant emissions of trace metals into the global environment. Subsequent atmospheric deposition of these metals resulted in many metal polluted sites worldwide. Trace metals accumulate in the soil and pose serious risks for ecosystems and public health through leaching to groundwater and dispersion in the food chain (WHO, 2000).

In the Campine (Kempen) region, situated in northeastern Belgium, zinc (Zn) and lead (Pb) were refined in three zinc smelters (Lommel, Balen, Overpelt) using a pyrometallurgical process from the end of the 19th century until the 1970s. Cadmium (Cd) occurs naturally in zinc, lead and copper ores and is usually produced as a byproduct of zinc refining. After World War II, Belgium became the principal producer of Cd in Europe (Lauwerys et al., 1990). In 1950, when metal processing was still very inefficient and poorly regulated, one of the zinc smelters in the area (Overpelt) emitted 340 kg Cd per day (Staessen et al., 1995). The Lommel zinc factory was dismantled in 1974, but the other two remain operational to this day. However, because of the transition from pyrometallurgical to electrolytic refining processes in the '70s, the termination of Pb-production and other technical innovations (e.g. filters), metal emissions have been reduced multifold during the past decades (Staessen et al., 1995; Wilkens and Loch, 1997; Seuntjens, 2002; Joris et al., 2014). In 2004, both remaining zinc smelters together emitted only 0.04 kg Cd per day (Peeters, 2006). Nevertheless, the high metal emission loads in the past and the use of residues (ashes, slacks, muffles) in roadways have resulted in a historical soil pollution of mainly Cd and Zn, and to a lesser degree of Pb, copper (Cu), arsenic (As) and mercury (Hg) (Ceenaeme et al., 2004; Joris et al., 2014). At present, primary emissions have been almost eliminated, whereas secondary sources of Cd and Zn due to resuspension of contaminated soil and road ashes are a major diffuse input to the area (Seuntjens, 2002; van der Grift and Griffioen, 2008). An extended area of about 700 km² in the Belgian and Dutch Campine region is now diffusely polluted (Van der Grift and Griffioen, 2008). Most of the metal contamination is found in the upper layer of the soil (up to 30-50 cm) but deeper soil layers and groundwater have become contaminated as well, due to leaching of the metals (Peeters, 2006; MIRA, 2010; Joris et al., 2014). Fig 1.1 clearly shows the (historical) Cd pollution around the three zinc smelters in the northern Campine region.

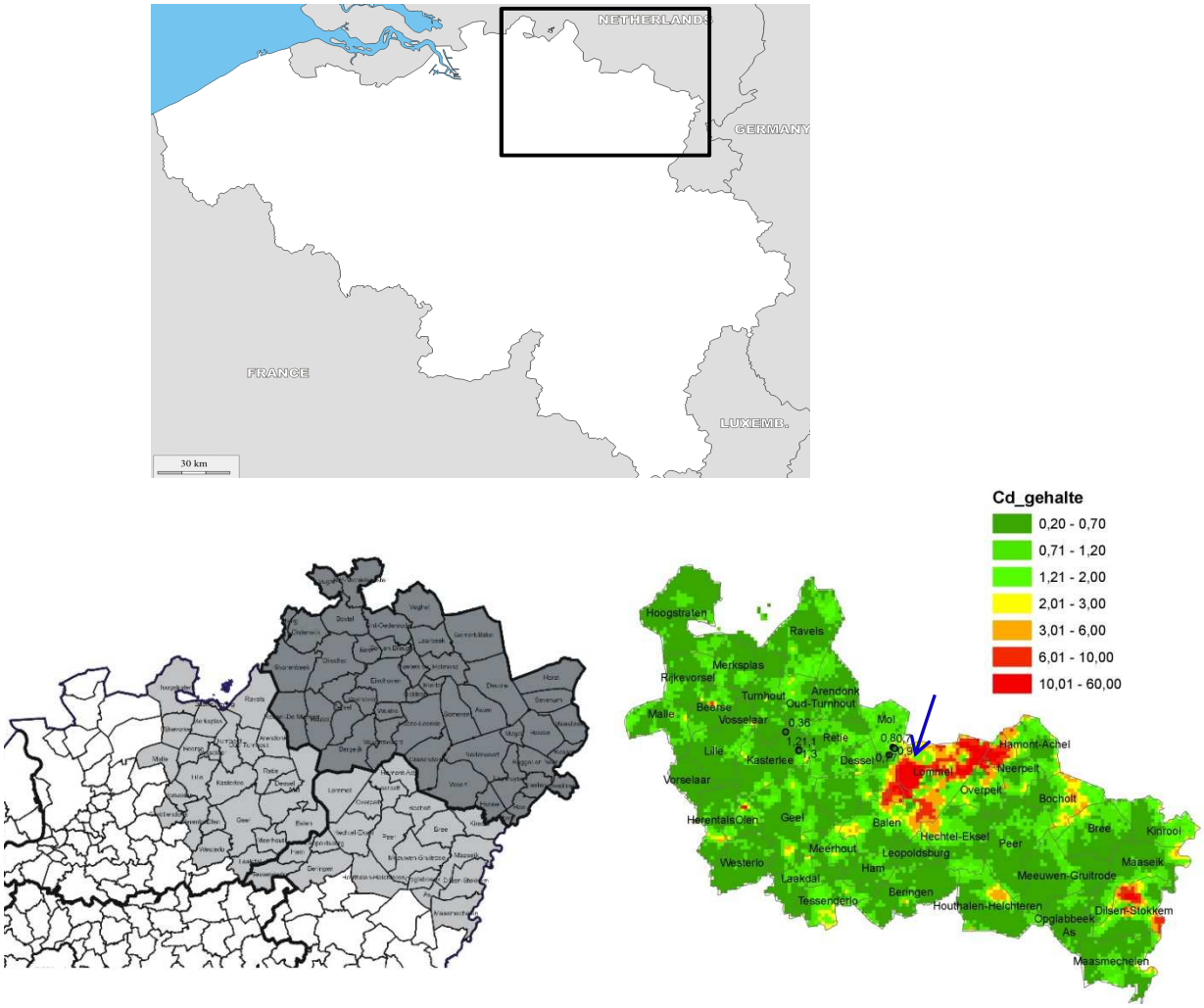

Figure 1.1 Location of the Belgian (light gray) and Dutch (dark gray) Campine region, with an overview of the Cd concentration (mg kg^{-1}) in the soil in the Belgian part of the region (OVAM, 2008). The location of our study site is indicated by the blue arrow (see Fig 3.1 for a detailed map of the study site)

Metal concentrations at an experimental site in the close vicinity (500 m) of the Balen smelter were found to be fairly elevated, with total Cd and Zn concentrations up to 12 mg kg^{-1} and 654 mg kg^{-1}, respectively (Van Slycken, 2011). However, apart from highly elevated metal concentrations in the immediate surroundings of the smelters, the soil contamination levels in the Campine region are moderate. As an example, total Cd concentrations in the upper 30 cm of the soil at our study site (Fig 1.1) ranged from 0.7 to 5.0 mg kg^{-1} (average 1.93 mg kg^{-1}), and total Zn concentrations in the upper 30 cm were between 35 and 350 mg kg^{-1} (average 116 mg kg^{-1}). Compared with the site-specific background concentrations and Flemish soil sanitation reference values (see Table 3.7), we found that the soil sanitation threshold of 1.59 mg Cd kg^{-1} was

exceeded in more than half of the sampling points at our study site. Zn concentrations in the majority of our sampling points were higher than the background concentration of 56 mg Zn kg^{-1}, but the soil sanitation threshold of 544 mg Zn kg^{-1} was not exceeded throughout our study site. Soil Cd concentrations at unpolluted sites in Europe and USA were found to be around 0.02 - 0.2 mg kg^{-1} (Holmgren et al., 1993; Andersen et al., 2002; Sevel et al., 2009). Benchmarking the metal concentrations measured at our study site against those from unpolluted sites on the one hand, and against those from the experimental site next to the smelter on the other hand, confirms that the soils in the Campine region are moderately contaminated.

The historical metal pollution in the Campine region still causes human-toxicological and ecotoxicological risks. Pb, As and Cd are a threat to human health, whereas Zn is phytotoxic. Cd in particular poses a serious health hazard as it is highly toxic and classified as a human carcinogen (IARC, 1993; Nawrot et al., 2006). Exposure to Cd occurs through intake of contaminated food or water, or by inhalation of tobacco smoke or polluted air; and Cd accumulates in the human body, particularly the kidneys (Järup et al., 1998). An important toxicological feature of Cd is its exceptionally long biologic half-life in the human organism, ranging from 10 to up to 30 years (Lauweryns et al., 1990; Nawrot et al., 2006).

During the '80s and '90s, two large-scale morbidity studies were carried out in the vicinity of the zinc smelters in the Belgian Campine region: Cadmibel (Cadmium in Belgium) and PheeCad (Public health and environmental exposure to Cadmium) (Hotz et al., 1999). It was shown that excessive exposure to Cd was linked to about 30% increased urinary cadmium excretion (Sartor et al., 1992), renal dysfunction (Buchet et al., 1990; Staessen et al., 1994), increased calciuria (Staessen et al., 1991), osteoporosis and a 35% population-attributable risk of fractures (Staessen et al., 1999). Moreover, Nawrot et al. (2006) found a significant association between risk of lung cancer and environmental exposure to Cd: residence in the high-exposure area was associated with a hazard ratio for lung cancer of 3.6. The latter study was breaking news in Belgium and headlined the national press (Fig 1.2). Above all, it raised a lot of questions and concerns among the people living in the northern Campine region, after which the Flemish government launched the 'Actionplan Cadmium' (Peeters, 2006). This project was aimed at determining the geographic extent of the Cd problem in Flanders and at giving an overview of possible actions to tackle the issue. One of the proposed actions was to plant fallow sites in order to reduce Cd spreading via wind erosion, and this way minimize Cd inhalation and ingestion by local residents. This concept, phytoremediation, will be discussed in detail below (see § 1.3).

KANKER DOOR CADMIUM

BRUSSEL. Voor het eerst is wetenschappelijk het verband vastgesteld tussen longkanker en cadmiumvervuiling.
Volgens een studie van drie Belgische universiteiten lopen inwoners van de Kempense gemeenten Overpelt, Lommel en Balen bijna vier keer meer kans op longkanker. Het gaat om mensen die wonen in de omgeving van een Umicore-vestiging. Dat gebied is historisch vervuild door cadmium.
Volgens dokter Jan Staessens, die het onderzoek leidt, is het verontreinigde gebied in de Noorderkempen groter dan tot nu werd aangenomen. Volgens een extrapolatie van zijn onderzoeksresultaten zijn wellicht 1.300 gevallen van longkankers rechtstreeks te wijten aan cadmiumvervuiling.

1.300 LONGKANKERS DOOR HISTORISCHE VERVUILING

Umicore wijst erop dat het al heel wat inspanningen heeft gedaan om de historische cadmiumvervuiling aan te pak
De afspraken die gemaakt voor de sanering van d briekstterreinen, zijn nage volledig uitgevoerd. Oo wijde omgeving van de Un re-vestigingen wordt g nigd.
Samen met de Openbar valstoffenmaatschappij (Ovam) legt Umicore de riteiten voor die sanering tegen juni dit jaar. Hiervo dertig miljoen euro opzij de helft is afkomstig va bedrijf. In 2002 is de ca umproductie stopgezet.
Inwoners van de veror nigde gebieden kunnen enkele eenvoudige hand gen de risico's beperkt ho Zo moeten omwonende woning stofvrij houden e met een vochtige doek w men; de handen wassen ze in de tuin hebben ge en het eten van groente zware metalen gemakkeli nemen, vermijden.

CADMIUM DIRECTE OORZAAK LONGKANKER

Volgens een extrapolatie hebben er in Noord-Limburg wellicht 1.300 mensen rechtstreeks longkanker opgelopen door cadmiumvervuiling.

Van onze redacteur Filip Verhoest

BRUSSEL. 'NEEN, wij konden de cijfers die nu bekend raakten, niet vlugger vrijgeven", zegt dokter Jan Staessens van de afdeling hypertensie van het Uni- monsters en urinestalen gebeurde twintig jaar geleden, maar men wacht tot nu om de resultaten mee te delen", verklaarde de burgemeester.
„De burgemeester van Lommel heeft er geen besef van wat epidemiologisch onderzoek inhoudt", zegt dokter Staessens. „Vijf jaar na de monsterneming was er nog geen statistisch significant verschil te zien en dan pas naar de media te stappen. Sommige collega's stappen eerst naar de media, en publiceren nooit een wetenschappelijk artikel."

VERONTREINIGD GEBIED GROTER DAN GEDACHT

Ze konden die mededeling maar weinig appreciëren. Men schoot op de boodschapper. Wij zijn tegengewerkt. Er werd zelfs gedreigd mijn onderzoekssubsidies af te nemen."

Burgemeesters horen niet graag zo'n onheilstijding?

„Neen. We mogen de onderzoeksresultaten nochtans niet onderschatten. In het verontreinigde gebied wonen zestig- tot vijfenzestigduizend mensen. Bij een groep van vijfhonderd mensen hebben wij over een periode van zeventien jaar vijftien longkankers aangetroffen. Als je die cijfers extrapoleert, weet je over hoeveel gevallen we spreken."
Het gaat dan om 1.950 longkankers in het verontreinigde gebied, tegen 650 in het (niet-vervuilde) controlegebied. Dat betekent dat 1.300 gevallen van longkankers rechtstreeks toe te schrijven zijn aan de cadmiumvervuiling.
Volgens Staessens is het verontreinigde gebied in Noord-Limburg ook groter dan tot nu is aangenomen. Daarenboven blijkt contact met cadmium via de luchtwegen even belangrijk als het eten van vervuilde groenten bijvoorbeeld.
„Dat betekent dat we de maatregelen voor preventie grondig moeten herdenken. Men zou de bodem meer kunnen vastleggen door begroeiing. Dan heb je minder opwaaiend stof."
„Om dezelfde reden mogen mensen in het verontreinigde gebied niet met stofzuigers, maar alleen met water schoonmaken."

➤ *Bladzijde 1: Kanker door cadmium.*

ONRUST NA KANKERSTUDIE

Inwoners van de Noorderkempen zijn ongerust, nu blijkt dat ze door cadmiumvervuiling een hoger risico op longkanker lopen.

BRUSSEL. „Veel mensen maken zich zorgen, en ik behoor daar ook toe", verklaarde de burgemeester van Overpelt, Karel Pinxten (VLD), gisteren op een persconferentie in Brussel, na een overlegvergadering met alle betrokkenen.
De Vlaamse minister van Leefmilieu, Kris Peeters (CD&V), zei dat hij begrip had voor de ongerustheid die bij de inwoners van Overpelt, Lommel en Balen leeft. Hij beklemtoonde niettemin dat de plaatselijke bevolking vooral belang heeft bij correcte informatie.
Volgens de studie van de universiteiten in Leuven, Louvain-la-Neuve en Hasselt lopen de inwoners van de wijken die bij de Umicore-vestigingen liggen, vier keer meer risico op longkanker dan in de rest van Vlaanderen *(DS 16 januari)*.
De wetenschappelijke waarde van het onderzoek van de universiteiten werd door de politici en ambtenaren niet openlijk ter discussie gesteld. Toch blijven er vragen onbeantwoord.
De burgemeester van Lommel bijvoorbeeld, Louis Van Velthoven (SPA), zei dat hij op de overlegvergadering van het Limburgs Kankerregister had vernomen dat er in Noord-Limburg niet significant meer longkankers voorkomen.
Die conclusie staat haaks op de bevindingen van de drie universiteiten. „De wetenschappers hebben nog een en

WETENSCHAPPERS MOETEN NOG EEN EN ANDER UITKLAREN

ander uit te klaren", luidde het commentaar van minister Peeters.
Een mogelijke verklaring, zei de Leuvense onderzoekers Tim Nawrot, is dat het Kankerregister een vergelijking tussen gemeenten maakt, terwijl de studie van de drie universiteiten de situatie ook heeft onderzocht in delen van gemeenten, bijvoorbeeld de woonwijken in de omgeving van de zinkfabrieken.
De gedelegeerd bestuurder van Umicore, Thomas Leysen, zei dat zijn bedrijf zijn verantwoordelijkheid voor de historische vervuiling niet wil ontlopen en de sanering van de wijdere omgeving van de Umicore-fabrieken zo snel mogelijk wil aanvatten. „We willen de volgende generatie niet met deze erfenis opzadelen", zei hij.
Voorlopig komen er geen bijkomende maatregelen voor sanering van de verontreinigde gebieden. Een beslissing hierover valt pas op 13 februari. „We moeten vermijden als kippen zonder kop te reageren", zei Peeters. (fvg)

➤ *Bladzijde 8-9: dossier cadmium en kanker.*

Figure 1.2 The study of Nawrot et al. (2006) about the association between environmental exposure to cadmium and cancer in northeast Belgium, published in The Lancet Oncology, became national headline news (De Standaard, January 2006)

In addition to the toxicological hazards associated with historical metal pollution, the risk for metal leaching and below-ground dispersion is aggravated by the predominant sandy texture of the soils in the region. Sandy soils are characterized by a low cation exchange capacity (CEC), low acid neutralizing capacity and low metal ion sorption ability (Andersen et al., 2002). This also implies that the metal availability for plant uptake is rather high (see § 1.2), which may result in an accumulation of metals in above-ground plant parts. This, in turn, may pose new risks for metal dispersion in the environment, through herbivores and litterfall. Therefore, remediation of the contaminated soils in the Campine region is necessary. However, due to the extent of the diffusely polluted area (700 km² spread over the Belgian and Dutch Campine region), conventional soil sanitation techniques are technically and financially inadequate to tackle the pollution problem. Hence, one should rely on risk based management of the area or on alternative remediation strategies. Examples of risk based management are: removal of zinc ashes, use of soil amendments to immobilize the metals in the soil, cultivation advices to farmers and to

the public (which vegetables are safe to grow), sensibilization campaigns to inform the public on how to reduce exposure to metals (use of groundwater for irrigation and drinking purposes, house cleaning tips, avoid bare soil in gardens, tips for playing children, …). Besides these risk based management options, phytoremediation has a lot of potential as an alternative remediation strategy. However, its practical applicability is still underexplored and should thus be further investigated. The phytoremediation technique will be discussed more in detail below (see § 1.3).

1.2 Behaviour of metals in soils

There is a consensus in the literature that total soil metal concentrations are not a good measure of metal bioavailability and thus not a very useful tool to determine potential environmental and human health risks (Sauvé et al., 2000a, 2000b). The evaluation of the potential risks and toxicity of metals in soils requires an assessment of the proportion of the total metal burden that is dissolved in the soil solution. Dissolved metals are mobile and could possibly be taken up by adjacent plant roots, be detrimental to various soil biological organisms, or otherwise be leached from the soil and contaminate groundwater and surface water (Sauvé et al., 2000a; Weng et al., 2001a). The concentration of metals in the soil solution, at any given time, is governed by a plethora of interrelated chemical reactions between the solid and the aqueous phase of the soil, including inorganic and organic complexation, oxidation-reduction reactions, precipitation-dissolution reactions and adsorption-desorption reactions (McLean and Bledsoe, 1992; Ross, 1994, McBride et al., 1997). These reactions are metal-specific and their relative importance depends on total metal content, on the concentration of organic and inorganic elements and ligands in the soil solution and on different soil properties, such as pH, CEC, organic matter dynamics and texture (e.g. Temminghoff et al., 1995; McBride et al., 1997; Römkens and Salomons, 1998; Sauvé et al., 2000a; Andersen et al., 2002; Voegelin et al., 2003).

A plethora of metal sorption studies have shown that solution pH has an overriding importance on metal solubility in many soils, as free metal ions and H^+ ions compete for adsorption on the soil's exchange sites (e.g. Christensen, 1984; Berthelsen et al., 1994; McBride et al., 1997; Sauvé et al., 2000a, 2000b; Strobel et al., 2001b, 2005; Andersen et al., 2002; Voegelin et al., 2003; Degryse et al., 2007). This is especially true for sandy soils with low CEC, low acid neutralization capacity, and low ability of the subsoil to sorb metal ions. Soils with higher contents of clay or organic matter (and thus a higher CEC) can counteract the acidification effect, as they have a higher metal adsorption capacity (Andersen et al., 2002).

Metal leaching can as well be stimulated by formation of soluble complexes with organic ligands, such as DOC (Sauvé et al., 2000b; Strobel et al., 2001b, 2005). Many authors found that soluble organic matter particularly has a strong affinity for Cu, Ni, Hg and Pb, whereas Cd and Zn tend not to complex strongly with soluble organics. This implies that the solubility of Cd and Zn is mainly controlled by pH with minor to no effects of DOC (e.g. Bergkvist et al., 1989; Holm et al., 1995; Römkens and de Vries, 1995; McBride et al., 1997; Sauvé et al., 2000b; Weng et al., 2001b; Strobel et al., 2001b, 2005).

Apart from the formation of organo-metal complexes, transport of metals through the soil matrix is also enhanced by formation of soluble complexes with inorganic ligands, such as SO_4^{2-}, Cl^-, PO_4^{3-}, NO_3^-. Cd and Zn tend to complex mainly with Cl^- and SO_4^{2-} (Benjamin and Leckie, 1982; Bergkvist et al., 1989; McLean and Bledsoe, 1992; Boekhold et al., 1993; Ross, 1994). Also the presence of base cations in the soil solution may significantly increase the mobility of some metals, as there is competition between the major cations and free metal ions for adsorption onto binding sites (Cavallaro and McBride, 1978; McLean and Bledsoe, 1992; Sauvé et al., 2000; Voegelin et al., 2003). Especially the competition between Ca^{2+} and Cd^{2+} has been described: increased Ca concentrations will displace Cd from sorption sites and mobilize Cd (Christensen, 1984; Temminghoff et al., 1995).

1.3 Phytoremediation

Phytoremediation is defined as the use of plants and their associated microorganisms to remove pollutants from the environment or to render them harmless (Salt et al., 1998). It depends on naturally occurring processes, in which plants detoxify inorganic and organic pollutants, via degradation, sequestration, or transformation (Pilon-Smits and Freeman, 2006). This green technology has gained popularity with government agencies and industry in the past 20 years (Pilon-Smits, 2005; Gomes, 2012) as it offers a low-cost alternative that retains the integrity of the soil and can be aesthetically pleasing (Pulford and Dickinson, 2005). Depending on the pollutant, substrate, and alternative remediation methods available, phytoremediation is typically 2- to 10-fold cheaper than conventional remediation techniques (Pilon-Smits and Freeman, 2006). Moreover, it creates environmental benefits such as soil erosion control, carbon sequestration and wildlife habitat (Gomes, 2012). All these advantages ensure a high degree of public acceptance for the phytoremediation technology (Pilon-Smits, 2005; Pulford and Dickinson, 2005; Gomes, 2012).

However, the use of phytoremediation also has some limitations. It may take an excessive amount of time for site remediation to be effective (see also Fig 2.1 and Table 2.1), and this time constraint is often considered to be the Achilles heel of phytoremediation (Robinson et al., 2003). Furthermore, with regard to metal pollution for instance, there are gaps in the knowledge about the potential risks related to metal dispersion in the environment, e.g. in food chains or via metal leaching to groundwater (Pulford and Dickinson, 2005; Mertens et al., 2007; Gomes, 2012). Metals may be transferred to more mobile and bioavailable forms when they return to the soil surface via litterfall, or deeper in the soil due to root or microbial action. In this way, phytoremediation could also provide a new exposure pathway for the metals (Perronnet et al., 2000; Robinson et al., 2003; Vervaeke et al., 2004; Pulford and Dickinson, 2005; Mertens et al., 2007). The scope of the phytoremediation process may be further limited by the plants' biomass production, their rooting depth, the (decreasing) bioavailability of the pollutants and the restricted number of target metals that can be detoxified at multi-metal contaminated sites (Robinson et al., 2003; Ernst, 2005; Pilon-Smits, 2005; Gomes, 2012). See Chapter 2 for a detailed discussion of all the limiting factors. Research suggests that phytoremediation is best suited for the treatment of slightly to moderately polluted areas and cannot serve as an equivalent for conventional soil remediation on more heavily polluted sites (Pulford and Watson, 2003; Dickinson and Pulford, 2005; Meers et al., 2010; Van Slycken, 2011).

Phytoremediation involves a variety of techniques and strategies that lead to contaminant degradation, removal (through accumulation or dissipation), transfer from or stabilization in soil and water (Pulford and Watson, 2003; Pilon-Smits and Freeman, 2006). Six main subtypes of phytoremediation have been identified: phytoextraction, phytodegradation, rhizofiltration, phytostabilization, phytovolatilization and rhizodegradation (= phytostimulation) (see Fig 1.3). The different phytotechnologies make use of different plant properties and typically different plant species are used for each (Pilon-Smits, 2005).

Inorganic pollutants (such as trace metals) are generally dealt with by phytoextraction and/or phytostabilization, while organic pollutants are most commonly treated by phyto- and rhizodegradation (Pilon-Smits and Freeman, 2006).

Both phytoextraction and -stabilization will be discussed in further detail in the following sections. The other four techniques are less relevant in the context of metal contaminated soils and were therefore considered to be outside the scope of this thesis.

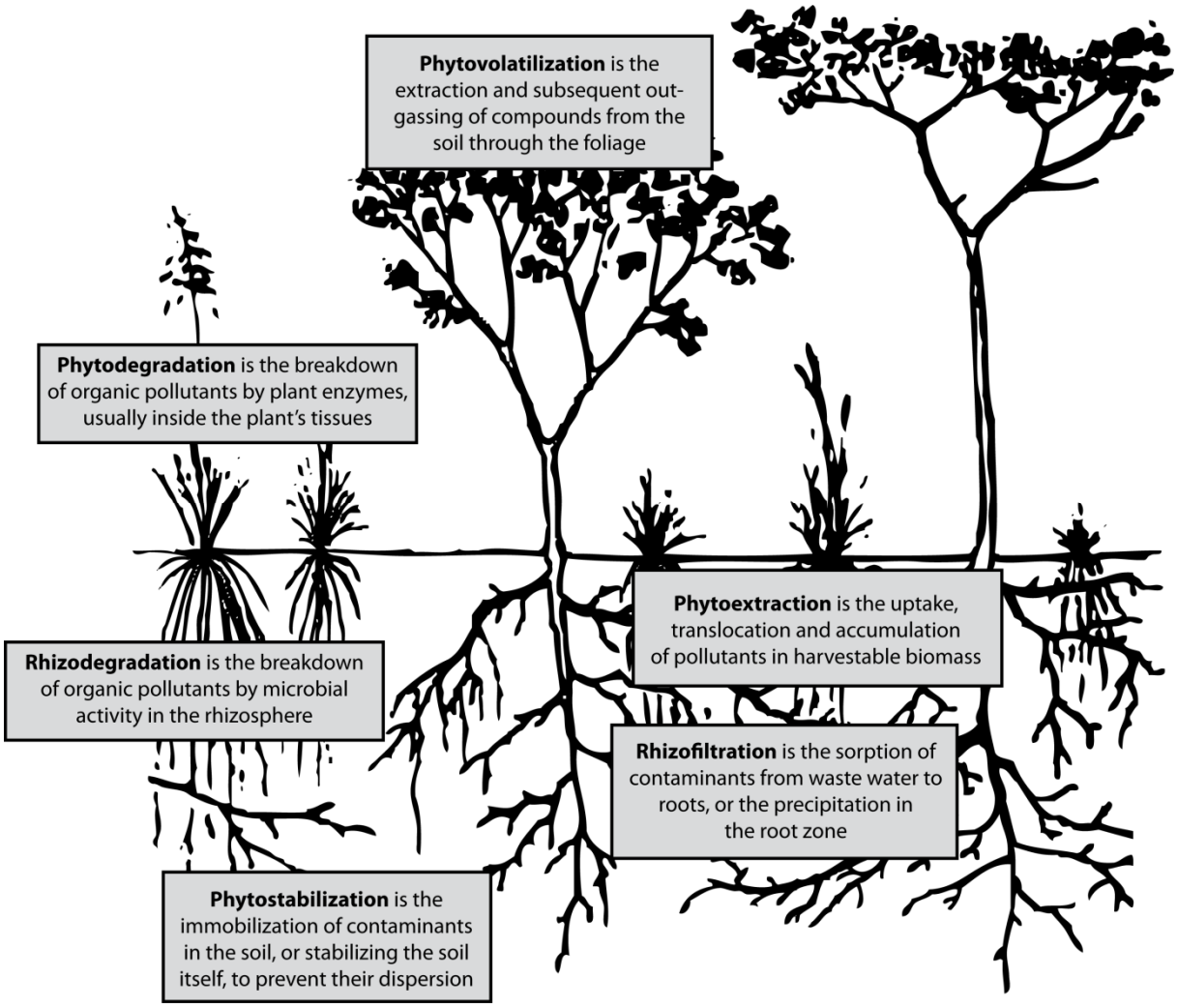

Figure 1.3 The main subtypes of phytoremediation (based on Pilon-Smits, 2005 and Gomes, 2012)

1.3.1 Phytoextraction

Phytoextraction aims at removing pollutants from the soil, by repeatedly harvesting plants that accumulate high levels of pollutants in their above-ground biomass. Fast growth rate, high biomass yield and high pollutant uptake and accumulation in harvestable tissues are thus important properties for plants used in phytoextraction projects. One category of plants that shows potential for phytoextraction of metal contaminated sites are the so-called hyperaccumulators, plants that accumulate toxic elements to levels that are at least 100-fold higher than non-accumulator species (Baker and Brooks, 1989; Peer et al., 2005). Hyperaccumulators have been reported for As, Co, Cu, Mn, Ni, Pb, Se and Zn (Baker et al., 2000; Ma et al., 2001). Despite these properties hyperaccumulators are not very popular for phytoextraction because they are often slow growers, attain low biomass and have no economic value (Pilon-Smits, 2005). Another possibility that is often referred to is the use of short rotation

coppice (SRC) for phytoextraction of metals from soils. SRC are carefully tended plantations of fast-growing trees for rotations shorter than 15 years (MacPherson, 1995). Although metal concentrations in trees are much lower than those in hyperaccumulators, total metal removal could be equal to those of hyperaccumulating plants because of their high biomass production (Pulford and Watson, 2003; Meers et al., 2007). Several authors postulated that trees have the ability to remove significant amounts of trace metals from the soil (Dickinson, 2000; Dickinson and Pulford, 2005; Meers et al., 2007). Willows (*Salix* spp.) and poplars (*Populus* spp.) are considered to be best suited for this task because of their strong ability to coppice, their high capacity for Zn and Cd uptake, and their high biomass production (e.g. Greger and Landberg, 1999; Robinson et al., 2000; Klang-Westin and Eriksson, 2003; Pulford and Watson, 2003; Rosselli et al., 2003; Laureysens et al., 2004; Vangronsveld et al., 2009).

One of the concerns inherent to the phytoextraction process is the treatment and/or disposal of the harvested, metal-rich biomass. The contaminated plant material might be used for non-food purposes (e.g. wood, cardboard) or it can be further concentrated by composting or ashing (producing bioenergy), followed by disposal in a landfill (Pilon-Smits and Freeman, 2006). Van Slycken (2011) investigated the conversion of metal contaminated energy maize (*Zea mays*) into biogas, through anaerobic digestion. The biogas production potential was similar to that of maize from an uncontaminated site, suggesting that the increased metal concentrations had very limited to no effects on the biomethanisation processes. The metal concentrations in the produced digestate were 3-4 times higher than those of the input material, yet the legal threshold values for using the digestate as a soil amendment were not exceeded. Sas-Nowosielska et al. (2004) examined various strategies (composting, compaction, incineration, ashing, pyrolysis, direct disposal and liquid extraction) and considered incineration (smelting) the most feasible, economically acceptable and environmentally sound. In the case of valuable metals, recycling of the accumulated element may be an option. This process, termed phytomining, is currently being used for nickel (Chaney et al., 2000).

Other concerns regarding the phytoextraction technology, and the risks, weaknesses and threats of phytoextraction practices in the field will be discussed in detail in Chapter 2.

1.3.2 Phytostabilization

Other than extracting metals from the soil, phytostabilization aims at stabilizing the metals in the soil, in order to avoid their above- and below-ground dispersion in the ecosystem. Phytostabilization can result from either physical or chemical effects. Trees can be very well

suited for phytostabilization purposes due to their large biomass, extensive root systems and high transpiration capacity (Pulford and Watson, 2003). The canopy layer, forest floor and roots form a 'green cap' which provides physical stabilization of the soil and decreases the risk of dispersion of metal contaminated soil particles by wind and water erosion (Vangronsveld et al., 1995a; Eviner and Chapin, 2003). In addition, litterfall adds significant amounts of organic matter to the (top)soil, promoting nutrient cycling, soil aggregation and water holding ability. Elevated evapotranspiration in forests decreases the downward soil water fluxes, and thus may reduce metal leaching losses to groundwater and surface waters (Pulford and Watson, 2003). Garten (1999) modeled the effect of a forest cover on Sr leaching from contaminated soil, mainly in shallow subsurface flow, and showed that the losses were reduced by approximately 16% under trees relative to grass. This observation was attributed to the greater evapotranspiration rate of the trees. Yet, even if only a grass cover is present, the leaching losses may also be reduced compared to bare soil. A percolation experiment, comparing waste dump substrate without and with 3-year-old grass cover, revealed that total amounts of percolated Cd and Zn were reduced by 84% and 87%, respectively, under the grass cover (Vangronsveld et al., 1995a).

On the other hand, tree growth may enhance metal mobility due to soil acidification, production of dissolved organic matter (Mayer, 1998), root-induced chemical changes in the rhizosphere (Nye, 1981; Hinsinger, 2000; Vervaeke et al., 2004) and eventual uptake of metals (Mertens et al., 2007). These effects are species-specific because tree species have different effects on soil parameters such as pH and organic matter content, and exhibit different element uptake patterns (Finzi et al., 1998; Augusto et al., 2002; Reich et al., 2005). Uptake and translocation of metals to the leaves might cause risks for above-ground metal dispersion in the environment. On the one hand, accumulated metals in the leaves may pose a long-term risk to primary consumers and enter the food web. On the other hand, contaminated litter may result in hot spots of available metals in the forest floor and in the topsoil. This could provide a new exposure pathway for the metals. The forest floor and topsoil are particularly vulnerable as they are the biologically most active parts of the soil system and biological activity has been shown to be highly sensitive to metal pollution (Bergkvist et al., 1989). Tree species inducing soil acidification will enhance metal solubilization (see § 1.2) and thus bear a potential risk for below-ground metal dispersion. Hence, with respect to risk control, it is very important to select tree species for phytostabilization purposes that cause low soil acidification and a minimal translocation of metals to their leaves (Mertens et al., 2007).

1.4 Objectives and general outline of the thesis

The success of phytoremediation projects is determined by the feedback mechanisms between the metal accumulation in trees, the leaf litter quality, the composition of the forest floor leachate and soil solution, and the evapotranspiration rate of the trees. These processes will affect metal mobility and are tree species specific. However, the global effects of trees on soil metals and the interspecific differences between tree species are still not fully investigated and understood. Therefore, this thesis studies the effects of different tree species on the fate of metals in the forest-soil system of afforested sandy soils. The study focuses on Cd and Zn because they are the main contaminants in the Campine region and are the most mobile and bioavailable of trace metals, implying that the risks of Cd and Zn dispersion in the ecosystem are higher compared to other metals. Determining the actual effects of Cd and Zn fluxes in the forest-soil system on public health and on ecological food webs, however, lies beyond the scope of this thesis.

The main objectives of this thesis are to

- investigate the biogeochemical and physical effects of six different tree species on Cd and Zn mobility on sandy soil.
- assess the feasibility of phytostabilization of diffusely Cd and Zn contaminated sandy soils by means of afforestation.

First, a conceptual approach of the actual and future applicability of phytoextraction and -stabilization is given (**Chapter 2**). After that, in order to further assess the feasibility of phytostabilization by afforesting diffusely contaminated sandy soils, an observational study was carried out in the Campine region in the northeast of Belgium. In this observational study, we first examine the general patterns involved in phytostabilization (**Chapter 3**). Six tree species were selected that are typical for the Campine sandy soils: silver birch (*Betula pendula*), oak (*Quercus robur* and *Q. petraea*), black locust (*Robinia pseudoacacia*), aspen (*Populus tremula*), Scots pine (*Pinus sylvestris*) and Douglas fir (*Pseudotsuga menziesii*). Subsequently, to get insight into the main patterns associated with phytostabilization, we aim to unravel the processes behind those patterns, under the six selected tree species (**Chapters 4-7**). See Fig 1.5 for a schematic overview of the thesis. Biogeochemical (mainly related to litter decomposition) and biophysical (evapotranspiration) processes play a central role in this study, as they are both known to be key players regarding metal mobility.

In **Chapter 2** we discuss the opportunities, threats and perspectives of practising the phytoextraction and phytostabilization techniques in the field.

Chapter 3 describes the tree species effects on soil properties that are decisive for metal mobility (pH, OC, CEC) and the effects on Cd and Zn uptake and redistribution in the soil profile after 10 years of tree growth.

Litter decomposition is one of the driving processes within the mobilization of metals, as it determines soil acidification and DOC leaching. Hence, in **Chapter 4**, litter decomposition dynamics and the accompanied changes in metal (Cd, Zn) and nutrient (base cations, N, C) amounts are quantified by means of a litterbag experiment. Tree species producing contrasting leaf litter in terms of chemical composition and degradability have a different influence on the composition and reactivity of forest floor leachate. Forest floor leachate chemistry in its turn determines to a large extent the biogeochemical processes in the soil and the soil solution chemistry, thus affecting metal mobilization and below-ground dispersion risks. Therefore, in **Chapter 5**, we explore the tree species effects on fluxes of Cd, Zn, DOC, H^+ and base cations in forest floor leachates, which were sampled with zero-tension lysimeters.

Sustainable phytostabilization requires the metals to be stabilized in the soil and should thus avoid above-ground (via uptake) and below-ground (via leaching) dispersion risks. However, typical tree species on the nutrient-poor sandy soils in the Campine region (e.g. pine, oak) are often characterized by nutrient-poor leaf litter, enhancing soil acidification and thus metal mobilization. Therefore, in **Chapter 6**, we investigate whether topsoil acidification in pine and oak stands can be counteracted by admixing shrub species with nutrient-rich leaf litter, such as European rowan (*Sorbus aucuparia*), alder buckthorn (*Rhamnus frangula*) and black cherry (*Prunus serotina*), and determine which is the threshold cover of the shrubs needed to obtain a significant effect in the topsoil.

Finally, to get insight into possible metal leaching to deeper soil layers and groundwater, the species-specific water and element seepage fluxes are quantified in **Chapter 7**. For this, we collected the soil solution with suction cup lysimeters at 50 cm depth and simulated the water fluxes at that depth by means of a water balance model.

1.4.1 Study sites

Chapters 3, 4, 5 and 7 were carried out in 'Waaltjesbos', a metal contaminated site on sandy soil in Lommel that was afforested between 1996 and 1998 and now serves as a public forest. Before afforestation, the site had been under agricultural use for at least several decades. The site is situated in between two zinc smelters, Lommel and Balen; the former was closed in 1974, while

the latter is still operational (see § 1.1). Detailed site descriptions are given in § 3.2.1. To unravel tree species effects on the behaviour of accumulated soil metals, Waaltjesbos is a unique study area since it originated from a rather homogenized situation (agricultural soil) which was subsequently planted with different tree species in blocks. The block pattern offers a perfect opportunity to study the tree species effects unambiguously. For each of the six considered tree species, three stands were selected throughout the forest (see Fig 3.1). The six tree species were chosen because they are typical for the Campine sandy soils and because they have divergent effects on metal uptake, on litter decomposition, on biogeochemical processes in the soil profile and on the water balance after plantation on an agricultural soil. Hence, Cd and Zn cycling under these tree species is expected to reflect the biogeochemical and biophysical interactions that took place over the preceding 10-15 years.

Chapter 6 was not carried out in Waaltjesbos, but in several uncontaminated forests in the Campine region (see § 6.2.1).

Understanding the aforementioned processes and their interactions is essential for sound phytoremediation that accounts for its possible risks. This thesis allows to gain fundamental insights into the driving factors of terrestrial metal cycling, and contributes to fill some of the knowledge gaps about the influence of tree growth and tree species choice on metal mobilization. This thesis may therefore become a useful tool for scientifically-based management of metal contaminated soils.

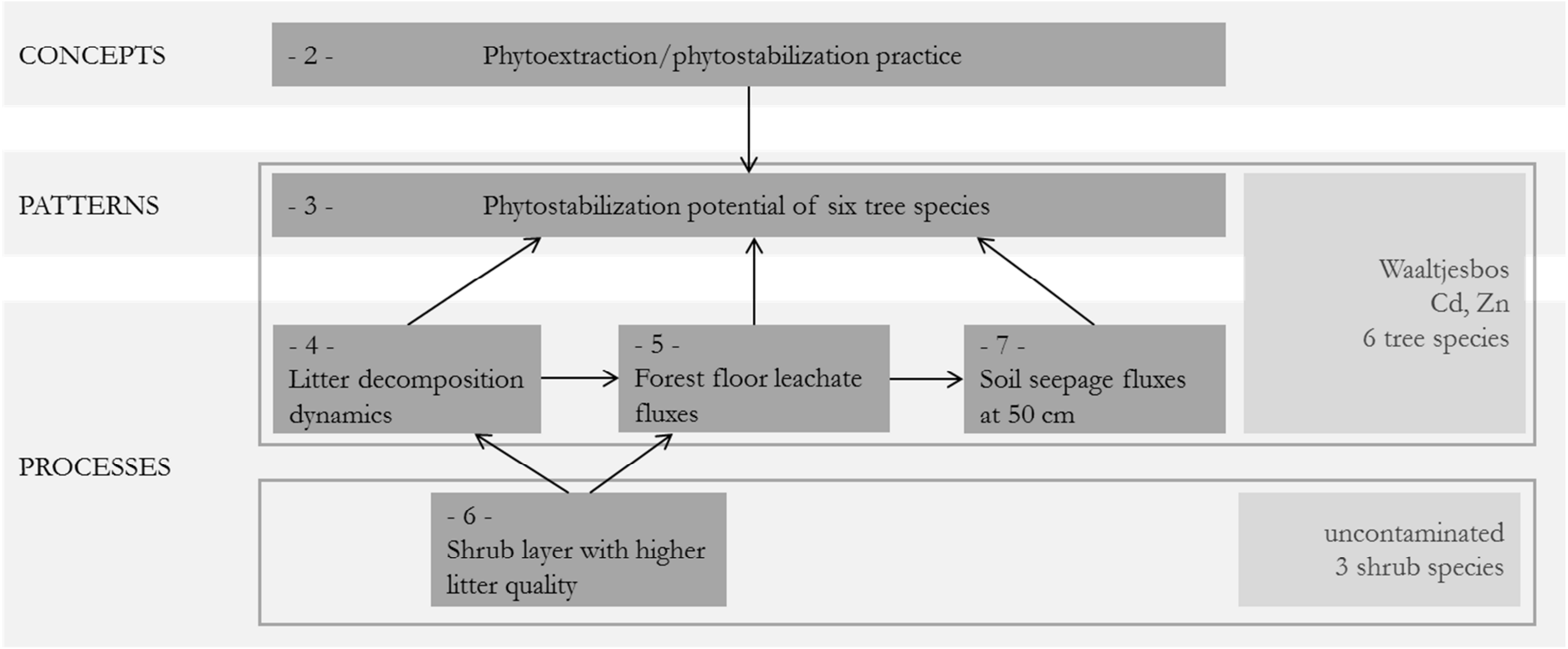

Figure 1.5 Schematic overview of the thesis with the interlinkages between the different chapters

2

Phytoextraction of metals from soils: how far from practice?

Abstract

For most trace elements, the technique of phytoextraction needs significant improvements to become practically feasible. Calculations for Cd revealed that the amount of Cd taken up by *Thlaspi caerulescens* or *Salix* spp. needs at least to be the double of the present amount to slightly decrease the Cd concentration in the upper 0.5 m of the soil within a period of 10 years. Additionally, metals taken up by the plants might pose an important risk. Alternatives as bioavailable contaminant stripping and phytostabilization might be more appropriate.

After: Van Nevel, L., Mertens, J., Oorts, K., Bogaert, G., Verheyen, K., 2007. Phytoextraction of metals from soils: how far from practice? Environmental Pollution, 150, 34-40.

2.1 Introduction

Phytoextraction is a phytoremediation technique that uses uptake by plants to remove metals and other contaminants from soils, sediments or water. Successful phytoextraction requires that the polluted medium is cleansed to a level that complies with environmental regulations, and from an economic viewpoint, this should be achieved at a lower cost than an alternate technology or the cost of inaction (Robinson et al., 2003). However, despite intensive research on the subject in the last decade, very few field trials or commercial operations that demonstrate successful phytoextraction have been realised (Robinson et al., 2006).

This leads us to the discussion on the opportunities and threats of phytoextraction practices in the field. In this paper we focus on the actual and future feasibility of phytoextraction of metals from polluted soils, giving attention to the risks involved in metal uptake by plants.

2.2 Metal phytoextraction on polluted soils: feasibility

Phytoextraction seems to be a simple and economic technique for the remediation of metal polluted soils. Nevertheless, ongoing research reveals that the applicability of the technique might be limited and that the practical implications might not be so evident as first thought. At present, the technology is limited by the long period required for cleanup, the restricted number of target metals that can be extracted, the limited depth that can be assessed by the roots, the difficulty of producing a high-biomass crop of the desired species and the lack of knowledge on the agronomic practices and management (e.g. Keller et al., 2003; Ernst, 2005; McGrath et al., 2006; Robinson et al., 2006). There is also concern about metal-accumulating plants providing an exposure pathway for toxic elements to enter the food chain if local herbivores consume these plants (e.g. Rock, 1997).

Robinson et al. (2006) simply state that phytoextraction is not ready for action. According to these authors, examples of successful phytoextraction are conspicuously absent. Also do Nascimento and Xing (2006) state in their literature review that it will take some time before phytoextraction may be established as a commercial technology. According to Peer et al. (2005), phytoextraction seems possible for As and Ni, while for the other metals the technology still appears to be far from practice. The vast majority of the hyperaccumulator species discovered so far are Ni hyperaccumulators (do Nascimento and Xing, 2006). Plant species that can accumulate Cd, Pb, Zn, Co, As and Cu are much less numerous (McGrath et al., 2001).

In a meta-analytical study, Audet and Charest (2007) concluded that phytoextraction efficiency will decline under increasing soil metal concentrations. According to Ernst (2005), phytoextraction is an option to decontaminate soils with low to moderate metal concentrations; for soils with a high load or with a deep penetration of metals, phytoextraction is not a realistic option. In those cases phytostablization is recommended instead.

2.2.1 Theoretical feasibility

But is phytoextraction indeed far from practice? As long-term site trials are not available, phytoextraction efficiency should be evaluated using models. Phytoextraction projects can be evaluated on the amount of metals being removed from the soil in relation to the total amount present in the soil (Mertens et al., 2005; 2006) and on the time needed for remediation.
To attain a predefined decrease of metal concentration in the soil profile over a certain depth, a certain metal amount has to be removed, that can be calculated by:

$$A = V_B \times d_B \times C_v \qquad (2.1)$$

Where A is the amount of metal to be removed per hectare (mg/ha), V_B the soil volume (m^3/ha), d_B the soil density (kg/m^3) and C_v the predefined concentration decrease (mg/kg).
Since the total extracted metal amount is the product of plant biomass and tissue metal concentration, the time needed to achieve the predefined soil concentration decrease is calculated by:

$$t = \frac{A}{P \times B} \qquad (2.2)$$

Where t is the time (yr), P is the crop metal concentration (mg kg^{-1}), and B is the annual biomass production (kg ha^{-1} yr^{-1}). Both P and B are spatial and temporal dependent as both parameters depend on the soil characteristics and available metal concentrations being spatial and temporal dependent (Robinson et al., 2006; see also further).

Here we discuss the theoretical feasibility of phytoextraction of Cd. To diminish the soil Cd concentration in the upper 0.50 m of the soil profile with 1 mg kg^{-1}, 8 kg Cd/ha (5000 m^3/ha x 1600 kg soil /m^3 x 1 mg Cd/kg soil) should be removed. To obtain a rough estimate, we assume that biomass production and crop metal concentration remain constant over time. The lines in Fig 2.1 represent the combinations of P, B and t that result in a removal of 8 kg Cd/ha according

to Eq. 2.2. The data points in this figure are literature data from field trials on medium polluted soils that reported crop concentrations as well as the biomass production (see Table 2.1).

Despite a theoretically possible reduction of 1 mg kg^{-1} in the soil's Cd concentration over a period of 15 years, it should be noticed that this is actually not very satisfactory in terms of **practice**. A remediation period of 15 years to achieve a decrease of 1 mg kg^{-1} might be too long to meet the demands of environmental decisionmakers. If we assume that a remediation period of 10 years is acceptable, we should conclude that none of the examples for slightly polluted soils (Fig 2.1) is able to attain a 1 mg kg^{-1} Cd reduction. The situation lies even further away if we have to attain a more profound concentration reduction. For the most polluted soil in Table 2.1, a decrease of 1 mg Cd kg^{-1} can be obtained in less than 2 years, but as the soil concentration is 163 mg kg^{-1}, this implies that the remediation period would be extremely long.

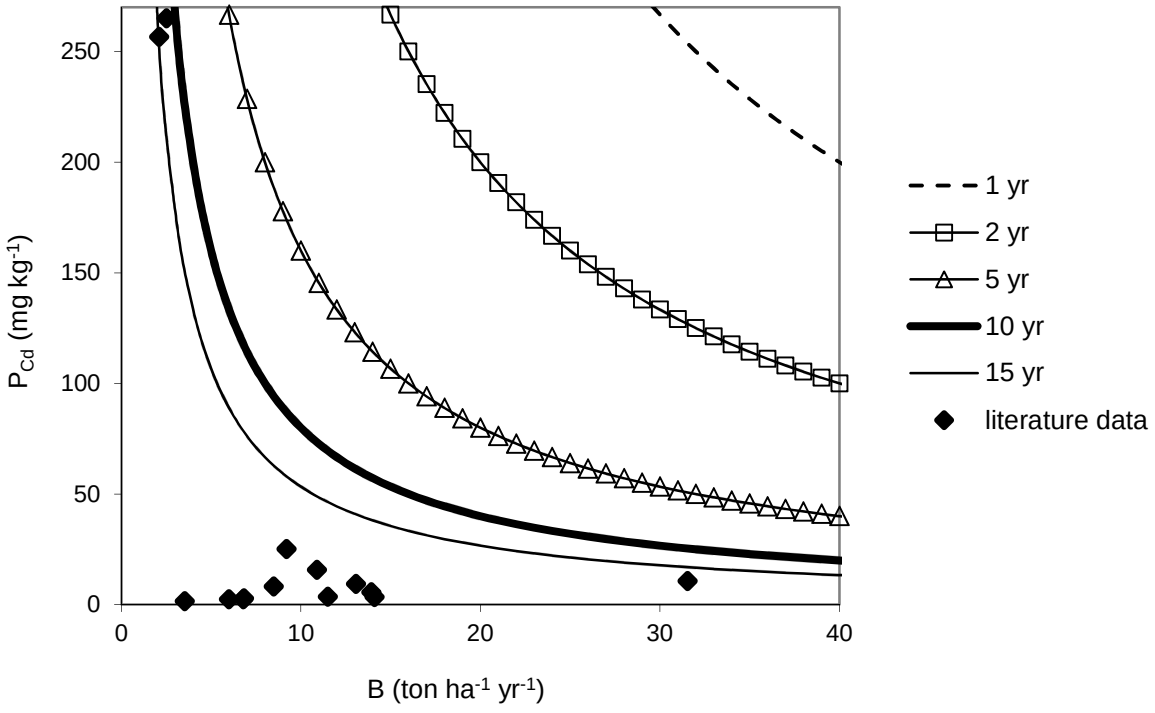

Figure 2.1 Annual biomass production (*B*) and crop Cd concentration (P_{Cd}) needed to decrease the Cd concentration in the upper 0.5 m of the soil with 1 mg kg^{-1} within different periods, assuming that *P* and *B* remain constant over time. P_{Cd} and *B* data retrieved from literature (see Table 2.1)

Table 2.1 Literature data reporting species, annual biomass production B, crop Cd concentration P_{Cd} and total Cd concentration in the soil. Calculated t is the time needed to remove 8 kg Cd/ha, calculated using Eq. 2.2

Species	B (ton ha^{-1} yr^{-1})	P_{Cd} (mg kg^{-1})	total soil [Cd] (mg kg^{-1})		Calculated t (yr)
Thlaspi caerulescens	2.6	1618	163 (1)	Robinson et al., 1998	1.9
T. caerulescens	2.1	256.7	2.8	Hammer and Keller, 2003	15
T. caerulescens	2.51	265	44	Maxted et al., 2003	12
Salix 'Calodendron' (*)	13.07	9.41	44	Maxted et al., 2003	65
S. dasyclados 'Loden' (*)	31.53	10.72	44	Maxted et al., 2003	24
Salix spp. (*)	9.2 - 13.9	5.6 – 25.2	4.6 - 5.0	Vervaeke, 2004	35 - 103
S. viminalis 'Orm' (*)	10.5 – 14.1	1.6 – 3.4 (**)	1.5 – 3.1 (2)	Meers et al., 2005	167 - 467
S. viminalis 'Orm' (2 yr) (*)	11.5	3.6	3 (2)	Vervaeke et al., 2003	193
S. viminalis (*)	2.1 - 8.7	0.6 – 4.1	0.17 - 0.45	Klang-Westin and Eriksson, 2003	471 - 1852
S. 'Calodendron' (*)	8.5	8.2	41.6 (3)	Maxted et al., 2007	115
Populus spp. (*)	3.5 – 6.0 (**)	1.6 – 2.4 (**)	0 – 7.9	French et al., 2006	563 - 1437
S. viminalis 'Orm' (*)	6.8 (**)	2.9 (**)	0 – 7.9	French et al., 2006	405

(*) *B* and *P* refer only to wood

(**) data derived from figures

(1) mine tailing, (2) dredged sediment, (3) sewage sludge

2.2.2 Why are basic calculations overestimates?

Due to temporal and spatial heterogeneity of the soil composition, this basic calculation certainly is a theoretical abstraction and an overestimate of the reality. As metals are removed from the soil by successive croppings, the **available metal concentration** will decrease. In some situations, the change may be approximately linear, however in most cases there could be a logarithmic decay upon successive annual croppings (Robinson et al., 1999). On dredged sediments planted with willow, a lesser proportion of total soil Cd was extractable by ammonium-acetate in a 6-year-old stand (6%) compared to a 1-year-old stand (17%) (Mertens et al., 2006). Long-term cropping of *Salix* resulted in a 30-40% decrease in plant-available Cd, although the effects on total Cd concentrations were negligible (Eriksson and Ledin, 1999). The efficiency of repeated croppings of the hyperaccumulator *Thlaspi caerulescens* to extract Cd and Zn from contaminated soils was examined by Keller and Hammer (2004). They measured a reduced Cd concentration already in the third cropping, indicating a decrease in Cd availability.

Since the metal concentration in an accumulator plant is correlated with the available concentration in the soil, the decreasing soil availability after several harvests will affect the metal yield of the crop, and hence the total extracted metal amount. However, it is doubtful whether the reduction of the bioavailable pool is definitive. Several authors described a replenishment of the bioavailable metal pool (Zhang et al., 1998; Whiting et al., 2001; Hammer and Keller, 2002; Keller and Hammer, 2004; Fischerova et al., 2006). This topic certainly needs further investigation as metal availability is crucial for the feasibility of phytoremediation.

Furthermore, the plants' **biomass production** might decrease in time, due to nutrient depletion in the soil after several croppings or pest infections. Fertilizers, pest control and crop rotation might be necessary. Plant growth may moreover be limited by other environmental variables, such as low pH, salinity, insufficient aeration or low water availability, whereas experimental conditions are generally optimal.

Finally, a lot of metal contaminated soils are polluted with more than one element, while few plant species can extract high concentrations of more than one element (Ernst, 2005). This **polymetallicity** might strongly affect the productivity of plants, even of metal resistant plants, causing the extraction period to become too long to be economically feasible (Marchiol et al., 2004; Ernst, 2005; Robinson et al., 2006). At such multi-metal contaminated sites, phytostabilization seems the most relevant technology in order to ensure a stabilization of the metals in the soil (Ernst, 2005).

Accounting for this expected decrease in phytoextraction efficiency, we end up even further from the possibility to decrease the soil Cd concentration with 1 mg/kg within a period of 10 years, than is seen in Fig 2.1.

2.2.3 How to perform more realistic estimates?

Ideally, long-term field trials are necessary, but are not practical for this objective. Therefore, validated mechanistic models are required that account for the spatial and temporal heterogeneity of metal distribution in the soil and for temporal changes of metal uptake by plants (Robinson et al. 2006). Although various aspects of vegetation-trace element interactions have been investigated in detail, there is, as yet, no quantitative model that integrates the aforementioned interactions (Robinson et al., 2006).

Robinson et al. (2006) rewrote Eq. 2.2, incorporating temporal and spatial heterogeneity of biomass production, crop metal concentration and soil concentrations:

$$t = \frac{M_i(x)_{\max} - M_f}{P(E) \times B(E)} \tag{2.3}$$

Where t is the time (yr), x is the spatial position (latitude, longitude), $M_i(x)_{\max}$ is the maximum initial metal burden (mg/ha), M_f is the target metal soil burden (mg/ha), P is the crop metal concentration (mg/kg DM), and B is the biomass production (kg DM/ha/yr). M_f will be highly dependent on the **depth of the rooting zone**, another limiting factor often overlooked in efficiency calculations. Both P and B are a function of the root exposure to bioavailable metal E (mg/kg). E can be calculated as:

$$E = \int_0^z \int_0^t R(t', z) C(M(t', z)) dt' dz \tag{2.4}$$

Where z is depth (m), R is the root fraction (-) that is in contact with the bioavailable metal C (mg/kg), which is a function of M.

It remains difficult to approach the temporal heterogeneity, since effects registered at short notice seldom reflect the long-term results. Koopmans et al. (2007) accounted for the decrease of total and available metal in the soil, estimating plant metal uptake by experimentally derived regression models describing the relationships between soil, soil solution and plant, instead of the assumption of constant plant uptake. Estimates of the duration of Cd phytoextraction using *T. caerulescens* - for their specific soil and assumptions - increased by more than 50 years. The regression models assumed equilibrium between metals sorbed to the soil solid phase and metals

dissolved in soil solution whereas in reality, replenishment of the soluble fraction is a slow process. This means that phytoextraction duration will last even longer than the estimates of Koopmans et al. (2007).

Moreover, roots in the field may not be in intimate contact with the contaminated material, thus resulting in lower-than-expected metal uptake (Keller and Hammer, 2004; Robinson et al., 2006). Schmidt (2003) demonstrated that the remediation efficiency of plants grown in the field was 20% lower compared to the plants under pot experimental conditions.

Phytoextraction projects could as well be evaluated on their cost effectiveness. The importance of the time needed for effective remediation, often hailed as the Achilles' heel of phytoextraction, might become less important if the cost of the operation is sufficiently low, or phytoextraction is combined with a profit making operation (e.g. phytomining, forestry or bio-energy production) (Robinson et al., 2003; Krämer, 2005). The viability of using phytoremediation in comparison to intensive remediation solutions or inaction can be assessed with tools as e.g. Decision Support Systems (DSS) (Robinson et al., 2003). However, Linacre et al. (2005) rightly emphasize the importance of likelihood of remediation success. They used a model that incorporates the factor 'uncertainty in project success', and illustrated that the possibility that full cleanup may not be realised may significantly increase the perceived costs of remediation works for decisionmakers. Therefore, modelling of uncertainty will be a key complement to models assessing the viability of phytoextraction from the scientific standpoint (Linacre et al., 2005).

2.3 Amelioration of the technique: perspectives?

As shown above, phytoextraction of most metals is still far from practice and the technique needs to be ameliorated. Most phytoextraction studies focus on enhanced plant metal uptake, by discovering or engineering new plants, and by soil amendments to enhance metal uptake.

2.3.1 Chelant-enhanced phytoextraction

To enhance metal uptake by plants, chelating agents that increase metal solubility are added to the soil. However, this technique contains some major drawbacks and environmental concerns, limiting the acceptance to use it in the field.

A large excess of chelant has to be applied to the soil due to the co-solubilization of Ca and Fe and in order to induce appreciable tissue concentrations (Nowack et al., 2006). At such soil solution concentrations, Nowack et al. (2006) found that plants will remove only a small fraction

of the solubilized metals. This causes leaching of both the chelant and solubilized metals, exacerbated by preferential flow processes, to be unavoidable. The use of chelators that significantly increases the risk of contaminant leaching, will do little to enhance the ability of phytoextraction to meet the demands of current environmental legislation (Robinson et al., 2006). Moreover, synthetic chelators are barely degradable by micro-organisms and thus persistent in the environment, posing adverse effects on soil microbiota (Bouwman et al., 2005).

The environmental concerns may limit the use of chelant-enhanced phytoextraction to applications where the connection to receiving waters has been broken, or where leaching is unimportant. In the former case, phytoextraction could be conducted ex situ. The contaminated material would be placed on a liner whereby any leachate could be collected and recycled (Nowack et al., 2006; Robinson et al., 2006).

Chelant-enhanced phytoextraction may nonetheless have a role in enhancing the uptake of essential trace metals. Such a role warrants further investigations into the use of biodegradable chelants such as ethylenediaminedisuccinic acid (EDDS) (Nowack et al., 2006; Meers et al., 2008). Application techniques could be adapted to minimize the risks of leaching.

2.3.2 Genetic manipulation

It is often claimed that genetically modified plants may have an advantage in phytoextraction compared to the wild species. However, genetic manipulation does not evidently contribute to solve the phytoextraction velocity. The engineering of transgenic plants suitable for phytoextraction will probably require a change in the expression levels of several genes. Beyond a certain number of genes, this could render transgenic approaches impractical (Krämer, 2005).

Genetic manipulation of plant rhizosphere, however, might be a good strategy, because it is thought that metal accumulator plants enhance metal solubility by releasing natural chelators from the roots (do Nascimento and Xing, 2006). This trail could furthermore overcome environmental constraints associated with chemically assisted phytoextraction. Insights into the effects of root exudates on metals are urgently necessary since this could have a dramatic impact on the feasibility of phytoextraction, either by using wild or transgenic plants (do Nascimento and Xing, 2006).

Finally, recent genome sequencing, the development of genomics tools, and the ease of genetic transformation of poplar might open up new avenues for the use of trees in phytoremediation (Krämer, 2005).

2.3.3 Bioavailable contaminant stripping

Hamon and McLaughlin (1999) introduced the concept of 'bioavailable contaminant stripping' (BCS). This technique is identical to phytoextraction but where phytoextraction aims at decreasing the total metal concentration, BCS aims the extraction of only the most labile, bioavailable metal pools. It is the available pool that causes environmental risks, and thus should be kept low enough to be harmless. This concept might be promising since cleanup time can be substantially shortened. However, in order to apply the technique efficiently and safely, it is necessary to assess the kinetics of replenishment of the bioavailable pool in the long term (Fitz et al., 2003).

2.4 Metal mobilization due to uptake: risks

Accumulation of metals in above-ground plant parts might cause risks for dispersion of metals in the environment. Three main risks can be identified: (i) metals entering the food chain through herbivores, (ii) dispersion of plant material to adjacent environments and (iii) accumulation of metals in the topsoil. Theoretical aspects of the risk analysis process in phytoextraction projects were recently reviewed by Linacre et al. (2003). The primary conclusion of this article was that risks of phytoextraction must be identified, quantified, managed, and communicated if the technology is going to find broad public acceptance.

2.4.1 Risks posed by metals in standing crop

The high metal concentration in the standing crop may pose a risk for herbivores. The risk of increased metal concentration in food for animals can be calculated based on a log logistic species sensitivity curve (Aldenberg and Slob, 1993). This is a cumulative frequency distribution of the critical metal concentrations in food (i.e. the highest concentration at which no negative effect was observed in laboratory feeding studies with contaminated foods) for various mammal and bird species (Fig 2.2). The critical concentration is usually defined as the 5% effect concentration, and for Cd in food this corresponds to 1.65 mg/kg fresh weight. At a crop Cd concentration of 20 mg/kg, it is predicted that already 71% of the animal species will be affected.

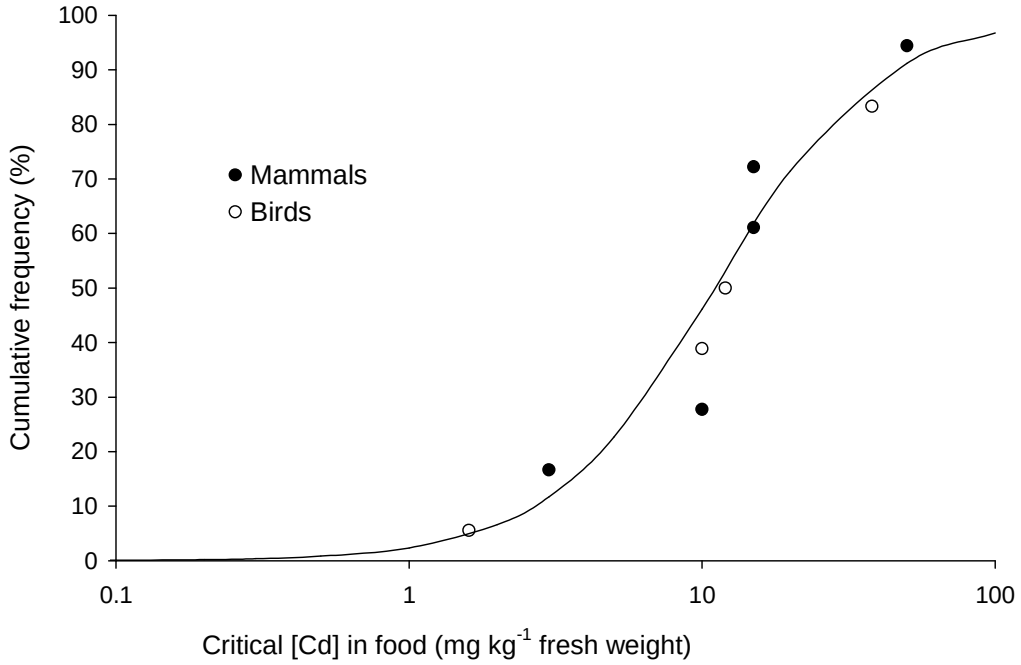

Figure 2.2 Species sensitivity distribution describing the risk of increased [Cd] in food for mammals and birds, based on existing data in literature (based on EU Risk Assessment Report for Cd, 2007)

This approach, however, assumes that the herbivores' diet completely consists of the contaminated food and therefore largely overestimates the risks. Some authors even report avoidance of plant material with high metal concentrations in the feeding behaviour of organisms (Boyd and Martens, 1992; Behmer et al., 2005). In order to correctly calculate the risk for herbivores, the proportion of contaminated leaves in the total diet of the herbivores should be known. Further, there is almost no information available on secondary poisoning through the plant-herbivore-predator pathway. Such information is critical for a correct assessment of the risks posed by the standing crops on the herbivorous organisms, and thus should be further investigated.

But not only the concentration in the standing crop might cause risks: *Pteris* spp. convert arsenate to arsenite (Peer et al., 2005). Although this conversion could make the arsenic less harmful for the plants, it is more harmful to animals and other organisms that might be exposed to the arsenite through plant contact.

2.4.2 Risks caused by litterfall

During growth and harvest, plant material might be dispersed, raising concern of contamination of adjacent environments (Perronnet et al., 2000). Also plant litter may be dispersed to adjacent areas through wind or water. When such plant material contains high metal concentrations, this may cause redistribution of the metals to unpolluted areas. Therefore, control measures should be taken in case dispersion with wind and water is expected to be important.

2.4.3 Accumulation of metals in the topsoil

The effect of plant biomass re-entering the soil can concentrate even more metals in the upper soil profile. Mertens et al. (2006) denoted that harvesting willows growing on polluted sediment after 4 or 6 years would result in more Cd distributed through foliar litterfall than Cd that could be removed through the harvest of the stems. Accumulation of metals in the leaves and foliar litterfall will cause a redistribution of metals in the soil. Metals taken up from the entire rooting zone will be concentrated in the upper soil horizons. In a field trial, it was seen that the concentrations of Cd and Zn in the upper soil layer under 33-years-old poplars were 2-3 times higher than under the even-aged oaks, ashes and maples (Mertens et al., 2007). Moreover, metals associated with organic matter are expected to be more mobile and bioavailable compared to metals adsorbed on mineral particles, because the organic matter decomposes in the soil, releasing the metals. Perronnet et al. (2000) reported that Cd and Zn associated with organic matter of *T. caerulescens* were highly available in soil. In their experiment, the leaves of *T. caerulescens* were incorporated into uncontaminated soil, whereafter Cd and Zn from this leaves exhibited a high mobility: Cd and Zn were transferred in large amounts to subsequent crops of rye grass (*Lolium perenne*) and *T. caerulescens*. About 94% of the Cd in rye grass and 86% in *T. caerulescens* originated from the incorporated leaves. Therefore, in natural environments, as well as in polluted areas, (hyper)accumulating plants may play a significant role in the metal cycle and generate hot spots of available metals in the upper part of the soil. This could provide a new exposure pathway for the metals, especially since the topsoil is highly vulnerable because the majority of biological life is concentrated there.

The risk of increased soil metal concentration for plants and invertebrates can be calculated in a similar way as described above for metal contaminated foods (Fig 2.3). Based on this curve, the percentage of species affected at a certain Cd concentration in the soil can be predicted. An increase of topsoil Cd concentration from 5 to 10 mg/kg will increase the potentially affected fraction of invertebrates and plants from 11 to 26 %. However, this prediction assumes that

organisms are only exposed to topsoil, which does not hold for the majority of species. No sufficient information is currently available to describe the effect of a local increase in metal concentrations on the overall risk for soil organisms. The risk of metal accumulation and increased metal bioavailability in the topsoil for the ecosystem health needs further investigation in order to properly assess the risks of phytoremediation techniques.

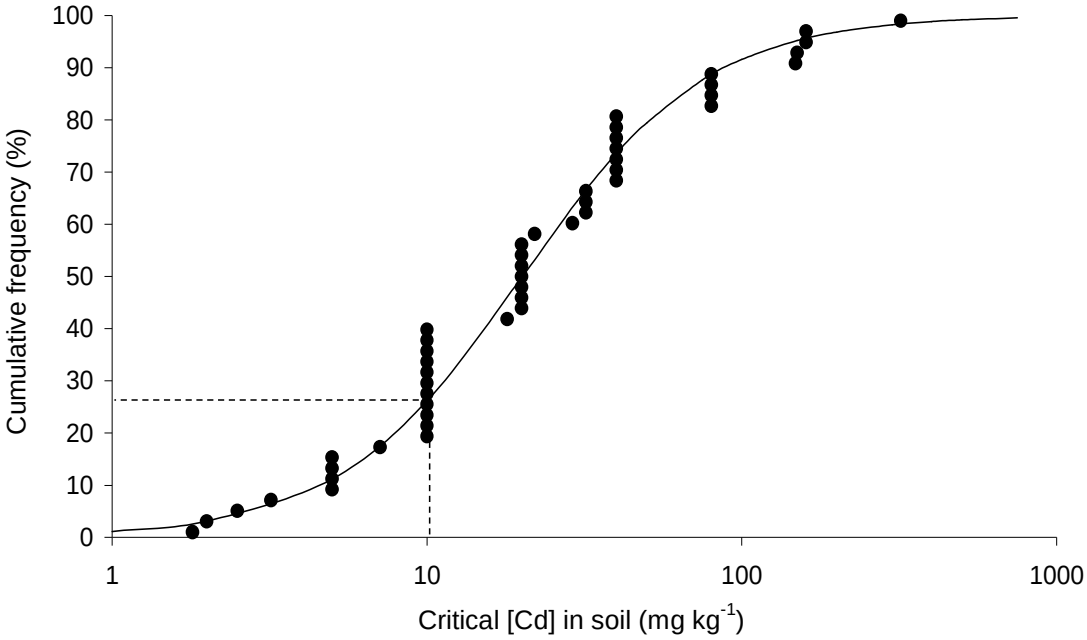

Figure 2.3 Species sensitivity distribution for the risk of increased soil [Cd] for invertebrates and plants, based on existing data in literature (based on EU Risk Assessment Report for Cd, 2007)

In case leaves and foliar litterfall contribute to a serious risk, harvesting the leaves in addition to the wood could be a possible management option to reduce the risk of food chain accumulation (Dickinson and Pulford, 2005).

At last, reducing the risk of contaminating the food chain or surrounding environments might be attained by means of **phytostabilization**. This technique aims at minimizing metal mobility using plants, and is a good alternative whenever phytoextraction is not possible or feasible. Trees are, due to their extensive root systems and high transpiration capacity, very well suited for phytostabilization purposes: they reduce leaching, control wind- and water erosion and add organic matter to the substrate that might bind the metals (e.g. Dickinson, 2000; Pulford and Watson, 2003). On the other hand, eventual soil acidification and production of dissolved organic matter might cause increased metal leaching (McBride et al., 1997; Sauvé et al., 2000a, 2000b;

Weng et al., 2002; Strobel et al., 2005). Hence, it is very important to select tree species for phytostabilization purposes that cause low soil acidification and do not translocate high amounts of metals to their leaves (Mertens et al., 2007).

2.5 Conclusions

Phytoextraction seems not practically feasible at the present state of knowledge. The technique needs significant ameliorations. Even then, applicability might be limited as metal uptake might cause risks for the environment. Models should be developed to predict site-specific feasibility of soil remediation using phytoextraction, and more research needs to be done regarding environmental risks of metal uptake by plants.

Reducing the risks of metal dispersion in the environment and contamination of the food chain may be attained by means of phytostabilization, as this technique aims at minimizing metal mobility. Although phytostabilization seems a more promising alternative for remediation of diffusely contaminated soils, this technique cannot guarantee to completely exclude all dispersion risks (see § 1.3.2). Moreover, its practical applicability is still underexplored and should thus be further investigated. The next chapters of this thesis contribute to answering these questions.

3

Elevated Cd and Zn uptake by aspen limits the phytostabilization potential compared to five other tree species

Abstract

Phytostabilization of metals using trees is often promoted although the influence of different tree species on the mobilization of metals is not yet clear. This study examined effects of six tree species on the soil characteristics pH, organic carbon (OC) content and cation exchange capacity (CEC) and on the redistribution of cadmium (Cd) and zinc (Zn) on a polluted sandy soil. Soil and biomass were sampled in 10-years-old stands growing on former agricultural land. The tree species included were silver birch (*Betula pendula*), oak (*Quercus robur* and *Q. petraea*), black locust (*Robinia pseudoacacia*), aspen (*Populus tremula*), Scots pine (*Pinus sylvestris*) and Douglas fir (*Pseudotsuga menziesii*). In the short period of ten years, only aspen caused significant changes in the soil characteristics. Due to accumulation of Cd and Zn in its leaf litter, aspen increased the total as well as the NH_4OAc-EDTA-extractable Cd and Zn concentrations in the topsoil compared to deeper soil layers and to other tree species. Also topsoil pH, OC content and CEC were significantly higher than under most of the other species. This caused rather low 'bioavailable' $CaCl_2$-extractable concentrations under aspen. Nevertheless, given the risks of above-ground metal dispersion and topsoil accumulation, it is recommended that aspen should be avoided when afforesting Cd and Zn contaminated lands.

After: Van Nevel, L., Mertens, J., Staelens, J., De Schrijver, A., Tack, F., De Neve, S., Meers, E., Verheyen, K., 2011. Elevated Cd and Zn uptake by aspen limits the phytostabilization potential compared to five other tree species. Ecological Engineering, 37, 1072-1080.

3.1 Introduction

Industrialization has effectuated significant emissions of trace metals into the global environment. Subsequent deposition of these metals and accumulation in the soil poses serious risks for ecosystems and public health through leaching of metals to groundwater and dispersion in the food chain (WHO, 2000). In the Campine region in northeastern Belgium, zinc (Zn) and lead (Pb) were refined from the end of the 19th century until the 1970s, resulting in an extended area of about 700 km² diffusely polluted by particularly cadmium (Cd) and Zn (Ceenaeme et al., 2004; Van der Grift and Griffioen, 2008). The risk for metal leaching and dispersion is intensified by the dominant sandy texture of the soils in the region. Sandy soils are characterized by a low cation exchange capacity (CEC), low acid neutralizing capacity and low metal ion sorption ability (Andersen et al., 2002).

Conventional soil remediation is technically and financially not feasible in this area because of the spatial extent of the metal pollution and the relatively moderate contamination levels. Therefore, adequate soil management that accounts for the present contamination appears to be the only realistic option. A possible management strategy for such degraded soils is afforestation (e.g. Dickinson, 2000; Pulford and Watson, 2003; Pilon-Smits and Freeman, 2006). In the Campine region, the most polluted agricultural soils have been taken out of production and a part of them has been afforested during recent years, in order to stabilize the metals and avoid their introduction in the food chain. This technique is also called phytostabilization.

Plants, and trees in particular, play an important role in the biogeochemical cycling of nutrients and pollutants, and can therefore be considered as ecosystem engineers (Jones et al., 1994). However, species-specific effects of plants on nutrient cycling are not yet adequately known (e.g. Reich et al., 2005; Hobbie et al., 2007), including the effect of trees on the biogeochemical cycling of metals. The main driving biogeochemical processes affecting metal mobility in soils are oxidation-reduction reactions, acidification, organic matter dynamics, and changes in base cation and anion concentrations and the CEC (McBride et al., 1997; Römkens and Salomons, 1998; Sauvé et al., 2000a; Andersen et al., 2002). The changes in the above-mentioned soil characteristics after afforesting agricultural land strongly depend on the tree species (Nordén, 1994; Alriksson and Eriksson, 1998; Augusto et al., 2002; Hagen-Thorn et al., 2004; Reich et al., 2005). Consequently, the distribution and fluxes of metals in biomass, litter and mineral soil will be species-specific as well (Alriksson and Eriksson, 2001; Watmough et al., 2005; Mertens et al., 2007). Selecting appropriate tree species is thus crucial for achieving successful phytostabilization. Trees can potentially be very well suited for phytostabilization purposes due to their extensive

root systems and high transpiration capacity (Pulford and Watson, 2003). On the other hand, tree growth might enhance metal leaching because of soil acidification and production of dissolved organic matter (Mayer, 1998). Hence, with respect to risk control, it is very important to select tree species for phytostabilization purposes that cause low soil acidification and a minimal translocation of metals to their leaves (Mertens et al., 2007).

Therefore, the aim of this study was to investigate the effects of six different tree species on (i) soil characteristics that influence metal mobility: pH, organic carbon (OC) content and CEC and (ii) on Cd and Zn compartmentalization after 10 years of tree growth on a sandy soil that was formerly under agricultural practice. We hypothesize that (i) tree species with high nutritional litter quality will ameliorate pH, OC content and CEC in the topsoil, whereas species with nutrient-poor litter will induce topsoil acidification and (ii) tree species which take up and translocate metals to their leaves will cause metal accumulation in the topsoil. However, given the young age of the forest, it is questionable whether these effects will be detectable already.

3.2 Materials and Methods

3.2.1 Site description and stand selection

The study site was the forest 'Waaltjesbos' (51°13'23" N, 5°15'01" E) in Lommel (northeast Belgium), which covers an area of 203 ha (Fig 3.1). The soil is a well-drained nutrient poor sandy soil (Podzol; IUSS WRB classification). The forest is located at the edge of the Campine plateau, which originates from a mixture of tertiary sands and gravel-rich sands deposited by the Meuse River. During the Pleistocene these sands were covered by aeolian sand deposits. The particle size distribution in the study site was determined as 92.3% sand, 2.0% silt and 3.3% clay.

The climate is sub-atlantic: the mean annual precipitation amounts to 800 mm and is evenly distributed throughout the year. The mean annual temperature is 9.0 °C (Royal Meteorological Institute of Belgium, http://www.kmi.be/).

The site was afforested between 1996 and 1998 and now serves as a public forest. Before afforestation, the site had been under agricultural use for at least several decades. Due to the past agricultural practices, there is a clearly visible (black) plough layer extending till 40 cm depth. Different tree species were planted in blocks, so that the forest consists of mainly homogeneous stands of pedunculate oak (*Quercus robur* L.), sessile oak (*Q. petraea* (Matt.) Liebl.), silver birch (*Betula pendula* Roth), Scots pine (*Pinus sylvestris* L.), Corsican pine (*Pinus nigra* ssp. *Laricio* Maire), black locust (*Robinia pseudoacacia* L.), Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), Japanese

larch (*Larix kaempferi* (Lambert) Carr.), black alder (*Alnus glutinosa* (L.) Gaertn.), common ash (*Fraxinus excelsior* L.) and beech (*Fagus sylvatica* L.). Aspen (*Populus tremula* L.) was planted in rows together with silver birch and black locust (30 – 50% aspen). No shrub layer is present in the forest. The block pattern offers a perfect opportunity to study the tree species effects unambiguously. No initial data were available on the soil characteristics and metal concentrations at the moment of the afforestation.

The site is situated in between two zinc smelters. Approximately 2 km north of the site used to be the former zinc smelter of Lommel, which was built in 1904 and was closed down and dismantled in 1974. The zinc smelter of Balen is about 2 km south-west, the predominant wind direction, of the site. It was built in 1889 and is still operational. Metal emissions from this factory have been controlled significantly and currently satisfy requirements according to European standards (see § 1.1). Nevertheless, the study site has been exposed to metal pollution during several decades, mainly to Cd and Zn and to a lesser degree to Pb, copper (Cu), arsenic (As) and mercury (Hg). The historical soil pollution in the region has two major causes: high metal emission loads in the past and subsequent atmospheric deposition on the one hand and application of zinc ashes for road construction on the other hand. Although these zinc ashes have been recently removed as part of decontamination policy in the region, possible remainders in the area cannot be excluded. The soil pollution in Waaltjesbos is diffuse and moderate, with Cd concentrations in the upper 30 cm of the soil up to 5.0 mg kg^{-1} (average 1.93 mg kg^{-1}), exceeding the soil sanitation threshold of 1.59 mg kg^{-1}. Zn concentrations in the upper 30 cm of the soil are up to 350 mg kg^{-1} (average 116 mg kg^{-1}), being higher than the background concentration of 56 mg kg^{-1} but not exceeding the soil sanitation threshold of 544 mg kg^{-1} (see Table 3.7).

In our study, six tree species were selected that are typical for the sandy soils in the study region and with potential to be used in future afforestations: oak (*Q. robur* and *Q. petraea*), silver birch, black locust, aspen, Scots pine and Douglas fir. For each of the considered tree species, three stands were selected throughout the forest to account for potential differences in soil characteristics, except for Douglas fir, for which only two stands were available (Fig 3.1). The size of the stands ranged from 0.5 to 4 ha. Stem diameters at 1.3 m height, stem numbers and basal area are shown in Table 3.1. Representative pictures of the stands are shown in Fig 3.2.

Figure 3.1 Map of Waaltjesbos, with the selected stands for each of the six tree species

Figure 3.2 Stand of silver birch (left) and Scots pine (right)

Figure 3.2 (continued) Stand of aspen (upper left), Douglas fir (upper right), oak (bottom left) and black locust (bottom right)

3.2.2 Sampling and sample analyses

Stem, branch and leaf litter biomass as well as different mineral soil layers were sampled in August 2007. At that time, the trees under study were 10 years old. In each selected stand, two healthy trees with average diameter were selected for sampling and marked as sampling points. Hence, six replicates were obtained per species, except for Douglas fir (four replicates). The sampling trees were cut and soil was sampled within a radius of 1 m, by pooling two core samples. On each felled tree, a stem slice was taken at 1.3 m stem height, while branches were collected throughout the tree crown. Leaf litter was collected with litterfall traps that had a circular surface area of 0.24 m^2. In every stand, four litterfall traps were emptied monthly in the autumn of 2007 until the broadleaved trees were leafless, and during one year for the conifer species (September 2007 – August 2008). Stem, branch and leaf litter samples were oven-dried at 70 °C to constant weight. Samples were not rinsed before drying. Every dried leaf litter sample was sorted out by hand to separate the leaf litter of the considered tree species while discarding other litterfall fractions. Monthly leaf litter samples were pooled per trap before analysis. Subsequently the biomass samples were milled and analyzed for Cd and Zn concentrations by inductively coupled plasma atomic emission spectroscopy (ICP-AES) after microwave destruction with an *aqua regia* solution (HNO_3/HCl).

Soil samples were taken with a soil auger at four fixed depths: 0-5, 10-20, 20-30 and 50-60 cm (below plough layer). The first three sampling depths were all entirely located in the Ap horizon, whereas the fourth sampling depth was located in the C horizon. Soil samples were oven-dried at 40 °C to constant weight. Aggregates were broken and soil was passed through a 2 mm sieve. Soil pH was determined in a 1:5 soil/KCl solution (1 M) with a glass electrode. The CEC was determined spectrophotometrically using a hexaminecobalt trichloride solution as extractant (ISO/DIS 23470). The OC content was determined spectrophotometrically by oxidation in a sulfochromic medium (ISO 14235). Pseudo-total Cd and Zn concentrations were determined by digesting the sediment for 2 h in an *aqua regia* solution under reflux (ISO 11466) and analysing the solution by ICP-AES. An estimation of the mobile Cd and Zn pool was made by determining their extractable concentrations in a 1:10 soil/$CaCl_2$ solution (0.01 M) and in a 1:10 soil/NH_4OAc-EDTA solution (0.5 M NH_4OAc and 0.02 M EDTA, set to pH 4.65) and subsequent ICP-AES analysis. The unbuffered $CaCl_2$ solution was used to extract exchangeable metals at ionic strength similar to that of the soil solution, whereas the NH_4OAc-EDTA solution was intended to extract a complexable metal fraction as well. Complexation by EDTA and acetic acid simulates the complexing behaviour by root exudates, whereas NH_4^+ ions are capable to

desorb the exchangeable soil fraction and the adapted pH simulates rhizosphere acidity (Meers et al., 2007).

3.2.3 Estimation of above-ground biomass

Estimation of the amount of above-ground biomass is necessary for calculating above-ground metal stocks. Leaf litter biomass was estimated by weighing the leaf litter collected in the litterfall traps (precision of 0.001 g). By summing the monthly litterfall masses, mean yearly dry biomass per litterfall trap was determined and extrapolated to annual leaf litterfall per hectare.

Woody biomass of stems and branches was assessed using species-specific allometric relations between tree diameter and dry stem or branch weight. The allometric relations were established for the studied forest stands according to the power function:

$$M = aD^b \qquad (3.1)$$

where M is the above-ground dry biomass per tree (kg), D is the stem diameter at 1.3 m height (cm), and a and b are allometric coefficients (Crow and Laidly, 1980; Zianis and Mencuccini, 2004).

To establish these relationships, D was measured for a random subsample of trees, spread over the three selected stands per species (Table 3.1), in order to define the diameter distribution per tree species. Then, trees were divided into four classes on the basis of the diameter range. For each D class one sample tree, representative of the class median, was felled in March 2008. For each sample tree, the branches were separated from the stem (needles were removed) and both compartments were separately weighed in the field by a portable scale with a precision of 10 g. Subsequently, a disc of approximately 10 cm thick was taken from the stem at 1.3 m height and weighed immediately on a portable scale to the nearest 0.1 g. A random subsample of the branches was weighed in the field as well (± 0.1 g). In the laboratory, all subsamples were oven-dried at 90 °C to constant mass, and weighed to recalculate the total dry biomass (M) of the stem and the branches of every sample tree. The established allometric relations (Table 3.1) were used to calculate the biomass of the stem and branches of every tree for which D was measured. Based on the stem numbers (Table 3.1), total stem and branches biomass was estimated per hectare for each tree species.

The Cd and Zn amounts in the stem, branches and leaf litter were calculated by multiplying the Cd and Zn concentrations with the estimated biomass.

Table 3.1 Stem diameter at 1.3 m height (*D*, average ± st.dev), stem number (N), basal area (BA) and parameters of the allometric relations (see Eq. 3.1, n = 4)

	D [§] (cm)	N [§] (ha^{-1})	BA [§] ($m^2\ ha^{-1}$)		*a*	*b*	R^2
silver birch	5.1 ± 2.2	3599	7.7	stem	0.0587	2.4881	0.996
				branches	0.0225	2.4326	0.998
oak	5.9 ± 1.9	4024	11.0	stem	0.1542	1.9986	0.982
				branches	0.0376	2.4163	0.927
black locust	9.3 ± 3.1	3551	24.8	stem	0.0849	2.4362	0.999
				branches	0.0055	2.8291	0.994
aspen	9.0 ± 3.2	4036	26.1	stem	0.12	2.1706	0.997
				branches	0.0202	2.52	0.994
Scots pine	7.7 ± 1.8	5010	23.4	stem	0.1175	2.006	0.957
				branches	0.0028	3.2158	0.991
Douglas fir	11.0 ± 2.5	2939	27.7	stem	0.1281	2.0278	0.966
				branches	0.0017	3.3768	0.985

[§] Based on measurements spread over the 3 stands per species, performed during winter 2007-2008

3.2.4 Data analysis

Differences between tree species were first tested using nonparametric analysis of variance (Kruskal-Wallis). Then, Mann-Whitney U tests were used to test the pairwise differences between the species. Moreover, soil data were subjected to an additional analysis to account for the spatial variation of the site. After all, both the historical atmospheric deposition as well as the possible remainders of the zinc ash roads may have contributed to a non-homogeneous metal pollution over the terrain before afforestation. The soil data indeed indicated an apparent spatial (horizontal) variation in soil characteristics and metal concentrations (e.g. total concentrations at 20-30 cm depth of 0.2 – 4.1 mg Cd kg^{-1} and 6.3 – 520 mg Zn kg^{-1}). On the other hand, the former agricultural use of the site has resulted in a strong (vertical) homogenization of the upper soil layers, due to many years of ploughing. Consequently we assumed a more or less uniform vertical distribution of the soil characteristics and metal concentrations in the soil profile until 40 cm depth at the moment of afforestation. After 10 years of tree growth, trees probably affected soil properties mostly in the upper soil layers. Therefore we considered the soil characteristics and metal concentrations in the 20-30 cm soil layer as being most representative for the initial site situation. Hence, to take into account the spatial variation, for each tree species the upper three soil layers (0-5, 10-20, 20-30 cm) were compared pairwise by means of a two-related-samples test (Wilcoxon signed-rank test). Correlations between pH, CEC, OC content, total and $CaCl_2$-

extractable metal concentrations were calculated for each soil layer by means of Spearman correlation coefficients.

One of the Scots pine sampling points was marked as an outlier, with exceptionally high values for all measured soil variables (e.g. pH-KCl 5.8, total concentrations 4.3 mg Cd kg^{-1} and 490 mg Zn kg^{-1} in the 0-5 cm layer), and was excluded from all data analyses. All statistical analyses were performed in SPSS 15.0. Following Moran (2003), the p-values were not corrected for multiple comparisons.

3.3 Results

3.3.1 Above-ground biomass: production and metal concentrations

Annual leaf litterfall was highest for Scots pine and aspen and lowest for silver birch and black locust (Table 3.2). Although no significant differences were found for the woody biomass estimates, black locust showed high stem wood production per ha. Silver birch produced low total above-ground biomass.

Table 3.2 Woody biomass estimates M_{stem} and $M_{branches}$, annual leaf litterfall M_{LF} and average wood production per year $Prod_{stem}$ and $Prod_{branches}$ for each tree species in 2008 (average ± st dev); values with the same letter did not differ between species ($p < 0.05$)

	M_{stem} (ton ha^{-1})	$M_{branches}$ (ton ha^{-1})	M_{LF} (ton ha^{-1} yr^{-1})	$Prod_{stem}$ (ton ha^{-1} yr^{-1})	$Prod_{branches}$ (ton ha^{-1} yr^{-1})
silver birch	19.0 ± 9.8 [a]	6.6 ± 3.3 [a]	1.4 ± 0.6 [a]	1.9 ± 1.0	0.7 ± 0.3
oak	26.2 ± 5.9 [a]	14.3 ± 3.9 [a]	3.7 ± 0.7 [b]	2.6 ± 0.6	1.4 ± 0.4
black locust	92.3 ± 28.2 [a]	15.6 ± 5.5 [a]	2.1 ± 1.0 [ac]	9.2 ± 2.8	1.6 ± 0.6
aspen	76.5 ± 27.7 [a]	29.7 ± 12.3 [a]	4.0 ± 1.0 [de]	7.7 ± 2.8	3.0 ± 1.2
Scots pine	41.7 ± 4.2 [a]	13.3 ± 2.2 [a]	4.9 ± 1.1 [e]	4.2 ± 0.4	1.3 ± 0.2
Douglas fir	56.6 ± 9.7 [a]	21.6 ± 4.1 [a]	3.1 ± 1.3 [bcd]	5.7 ± 1.0	2.2 ± 0.4

Significant differences between the six tree species were found for both Cd and Zn concentrations in the above-ground biomass compartments (Table 3.3). Aspen clearly took up more Cd than the other species, with the Cd concentration in the leaf litter being about 7 times higher than that of the other tree species. Zn concentrations in all the compartments were significantly higher in silver birch and aspen. Among the other species, only minor differences in Cd and Zn concentrations were found.

The amount of Cd and Zn in the above-ground biomass of aspen was clearly higher than for the other tree species (Table 3.4). The above-ground Zn pools in silver birch, on the other hand, were not elevated compared to the other species, despite high Zn concentrations in its biomass (Table 3.3). This was caused by its low biomass production (Table 3.2).

Table 3.3 Cd and Zn concentrations (average ± st dev) in the biomass compartments; per compartment, values with the same letter did not differ between species ($p < 0.05$)

	Cd (mg kg^{-1})	Zn (mg kg^{-1})
	Stem	
silver birch	0.83 ± 0.38 (3) [a]	239 ± 75 [c]
oak	< 1.0 [a]	53 ± 31 [a]
black locust	< 1.0 [a]	56 ± 15 [a]
aspen	2.72 ± 1.50 (1) [b]	146 ± 60 [b]
Scots pine	1.03 ± 0.49 (2) [a]	63 ± 34 [a]
Douglas fir	< 1.0 [a]	41 ± 15 [a]
	Branches	
silver birch	1.88 ± 0.81 [b]	701 ± 161 [b]
oak	< 1.0 [a]	171 ± 53 [a]
black locust	< 1.0 [a]	180 ± 30 [a]
aspen	7.02 ± 2.66 [c]	497 ± 181 [b]
Scots pine	1.70 ± 0.22 [b]	177 ± 59 [a]
Douglas fir	0.87 ± 0.43 (2) [ab]	144 ± 23 [a]
	Leaf litter	
silver birch	1.72 ± 0.44 [c]	1 540 ± 341 [c]
oak	< 1.0 [a]	287 ± 106 [a]
black locust	1.22 ± 0.40 (1) [bc]	427 ± 100 [b]
aspen	13.0 ± 6.5 [d]	2 354 ± 494 [d]
Scots pine	1.08 ± 0.34 (1) [b]	321 ± 56 [a]
Douglas fir	< 1.0 [a]	295 ± 22 [a]

< if all values were lower than the determination limit, the determination limit was given, preceded by '<'

(1), (2), (3) the number between brackets is the number of values lower than the determination limit of 1.0 for Cd. The mean was calculated by equalling those values to the half of the determination limit.

Table 3.4 Cd and Zn amounts (average ± st dev) in woody biomass and annual leaf litterfall of the six tree species

	Cd ($g\ ha^{-1}$)	Zn ($g\ ha^{-1}$)
	Stem	
silver birch	16.2 ± 7.6	4 662 ± 1 533
oak	< 26.7	1 422 ± 852
black locust	< 91.2	5 124 ± 1 427
aspen	208.2 ± 115.6	11 188 ± 4 666
Scots pine	44.3 ± 23.2	2 723 ± 1 592
Douglas fir	< 57.4	2 338 ± 947
	Branches	
silver birch	12.6 ± 5.5	4 688 ± 1 117
oak	< 15.8	2 701 ± 1 395
black locust	< 15.5	2 775 ± 570
aspen	209.7 ± 82.7	14 845 ± 5 649
Scots pine	23.2 ± 4.8	2 409 ± 896
Douglas fir	18.7 ± 9.9	3 086 ± 769
	Leaf litter	
silver birch	2.4 ± 1.3	2 159 ± 1 093
oak	< 3.7	1 063 ± 438
black locust	2.5 ± 1.5	893 ± 492
aspen	52.4 ± 29.5	9 461 ± 3 146
Scots pine	5.3 ± 2.0	1 562 ± 452
Douglas fir	< 3.1	910 ± 402

3.3.2 Soil

pH, OC and CEC

Only under aspen the pH in the topsoil (0-5 cm) was significantly increased compared to the deeper soil layer and to the other tree species (Table 3.5). At 10-20 cm depth the pH under silver birch was lower than under aspen, Scots pine and Douglas fir. With respect to the OC content in the topsoil, two groups could be distinguished, namely aspen and black locust in the higher range and the other species with lower OC contents. Aspen showed a significantly higher OC content in the topsoil compared to deeper layers. The CEC in the upper 5 cm of soil under aspen was higher than for the other species, whereas the lowest CEC values were found under silver birch and oak. Aspen had induced a CEC increase in the topsoil compared to 10-20 cm depth, whereas the CEC under silver birch at 10-20 cm depth was lower than at 20-30 cm depth. In each soil layer the CEC was significantly ($p < 0.05$) correlated with both pH (Spearman's $\varrho = 0.381$ - 0.692) and OC content ($\varrho = 0.341$ - 0.687), while pH and OC content were not significantly correlated ($p > 0.06$, $\varrho < 0.374$). Pairwise comparison revealed that pH, OC and CEC in the deepest soil layer (50-60 cm) were significantly lower than at 20-30 cm depth (significances not shown).

Table 3.5 Soil characteristics pH-KCl, OC and CEC at four depths (average ± st dev); values followed with the same letter did not differ, first capital letters denote species effects and should be read vertically ($p < 0.05$), second small letters denote differences between soil layers and should be read horizontally ($p < 0.05$)

	0 – 5 cm	10 – 20 cm	20 – 30 cm	50 – 60 cm
		pH-KCl (-)		
silver birch	4.5 ± 0.3 $^{A\,a}$	4.3 ± 0.2 $^{A\,a}$	4.5 ± 0.1 $^{A\,a}$	4.4 ± 0.2 ^{A}
oak	4.4 ± 0.2 $^{A\,a}$	4.5 ± 0.3 $^{AB\,a}$	4.6 ± 0.3 $^{A\,a}$	4.7 ± 0.3 ^{A}
black locust	4.4 ± 0.3 $^{A\,a}$	4.5 ± 0.3 $^{AB\,a}$	4.7 ± 0.3 $^{A\,a}$	4.5 ± 0.1 ^{A}
aspen	5.4 ± 0.6 $^{B\,b}$	4.7 ± 0.3 $^{B\,a}$	4.8 ± 0.5 $^{A\,ab}$	4.8 ± 0.4 ^{A}
Scots pine	4.5 ± 0.3 $^{A\,a}$	4.7 ± 0.3 $^{B\,a}$	4.8 ± 0.4 $^{A\,a}$	4.5 ± 0.4 ^{A}
Douglas fir	4.4 ± 0.4 $^{A\,a}$	4.7 ± 0.1 $^{B\,a}$	4.7 ± 0.2 $^{A\,a}$	4.4 ± 0.2 ^{A}
		OC (g C kg^{-1})		
silver birch	19 ± 5 $^{A\,a}$	14 ± 6 $^{A\,a}$	14 ± 6 $^{A\,a}$	7 ± 4 ^{A}
oak	17 ± 1 $^{AB\,a}$	18 ± 5 $^{A\,a}$	16 ± 4 $^{A\,a}$	11 ± 8 ^{A}
black locust	32 ± 9 $^{BC\,a}$	15 ± 8 $^{A\,a}$	18 ± 8 $^{A\,a}$	6 ± 3 ^{A}
aspen	29 ± 6 $^{C\,b}$	18 ± 9 $^{A\,a}$	17 ± 7 $^{A\,a}$	13 ± 7 ^{A}
Scots pine	20 ± 7 $^{AB\,a}$	16 ± 7 $^{A\,a}$	14 ± 8 $^{A\,a}$	5 ± 4 ^{A}
Douglas fir	17 ± 4 $^{AB\,a}$	19 ± 7 $^{A\,a}$	14 ± 1 $^{A\,a}$	12 ± 12 ^{A}
		CEC (cmol kg^{-1})		
silver birch	2.8 ± 1.2 $^{A\,ab}$	2.2 ± 0.8 $^{A\,a}$	2.9 ± 1.1 $^{A\,b}$	1.7 ± 0.4 ^{A}
oak	2.8 ± 0.6 $^{AC\,a}$	2.7 ± 0.7 $^{AC\,a}$	3.1 ± 0.7 $^{AC\,a}$	2.8 ± 1.7 ^{AB}
black locust	5.8 ± 1.0 $^{BCD\,a}$	4.5 ± 0.9 $^{B\,a}$	4.6 ± 0.7 $^{B\,a}$	3.4 ± 0.6 ^{B}
aspen	8.1 ± 1.7 $^{D\,b}$	4.8 ± 1.8 $^{BC\,a}$	4.9 ± 1.5 $^{BC\,ab}$	4.0 ± 1.8 ^{B}
Scots pine	4.7 ± 0.7 $^{B\,a}$	4.6 ± 0.8 $^{B\,a}$	4.2 ± 1.1 $^{AB\,a}$	2.8 ± 0.7 ^{B}
Douglas fir	4.6 ± 1.0 $^{AB\,a}$	3.9 ± 0.2 $^{B\,a}$	3.7 ± 0.4 $^{AB\,a}$	2.8 ± 1.5 ^{AB}

Cd and Zn concentrations

Total Cd and Zn concentrations in the topsoil were highest under aspen and differed significantly from the lower Cd and Zn levels under the other species, except for Scots pine (Table 3.6). For aspen the total metal concentrations in the topsoil were significantly higher than those in deeper layers. The tree species effect was most pronounced in the upper 5 cm, as no differences between species were found in the deeper soil layers.

In contrast to total soil Cd and Zn, the $CaCl_2$-extractable concentrations were not highest in the topsoil of aspen but of Scots pine, although no significant differences between the species or the soil layers were found. NH_4OAc-EDTA-extractable concentrations, however, followed the same trends as total concentrations, with significantly higher values in the topsoil under aspen compared to other species and to deeper soil layers. In addition, NH_4OAc-EDTA-extractable Cd under oak was significantly higher at 10-20 cm than in the topsoil.

Pairwise comparison revealed that both total and extractable Cd and Zn concentrations in the deepest soil layer (50-60 cm) were significantly lower than at 20-30 cm depth (significances not shown).

Table 3.6 Total and extractable soil Cd and Zn concentrations (mg kg^{-1} DM) at four depths (average ± st dev); values followed with the same letter did not differ, first capital letters denote species effects and should be read vertically ($p < 0.05$), second small letters denote differences between soil layers and should be read horizontally ($p < 0.05$)

	0 – 5 cm	10 – 20 cm	20 – 30 cm	50 – 60 cm
		Cd		
silver birch	1.42 ± 0.86 (1) $^{AB\,a}$	1.50 ± 0.72 (1) $^{A\,a}$	1.67 ± 1.02 (1) $^{A\,a}$	0.42 ± 0.40 (4) ^{A}
oak	1.45 ± 0.42 $^{A\,a}$	1.96 ± 0.92 $^{A\,a}$	1.90 ± 0.76 $^{A\,a}$	0.83 ± 0.98 (4) ^{A}
black locust	1.70 ± 0.70 $^{AB\,a}$	2.15 ± 1.12 (1) $^{A\,a}$	1.82 ± 1.22 (1) $^{A\,a}$	0.43 ± 0.38 (4) ^{A}
aspen	3.55 ± 1.44 $^{C\,b}$	2.20 ± 1.30 (1) $^{A\,a}$	1.96 ± 1.14 (1) $^{A\,a}$	1.15 ± 0.97 (2) ^{A}
Scots pine	2.34 ± 0.67 $^{BC\,a}$	1.88 ± 0.59 $^{A\,a}$	1.75 ± 0.93 $^{A\,a}$	0.33 ± 0.20 (4) ^{A}
Douglas fir	1.55 ± 0.29 $^{AB\,a}$	1.88 ± 0.72 $^{A\,a}$	1.88 ± 0.34 $^{A\,a}$	0.33 ± 0.26 (3) ^{A}
		Zn		
silver birch	87 ± 52 $^{AB\,a}$	73 ± 44 $^{A\,a}$	77 ± 40 $^{A\,a}$	30 ± 19 ^{A}
oak	86 ± 29 $^{A\,a}$	109 ± 68 $^{A\,a}$	110 ± 56 $^{A\,a}$	51 ± 47 ^{A}
black locust	123 ± 62 $^{AB\,a}$	135 ± 70 $^{A\,a}$	120 ± 72 $^{A\,a}$	29 ± 23 (1) ^{A}
aspen	267 ± 91 $^{C\,b}$	120 ± 72 $^{A\,a}$	114 ± 72 $^{A\,a}$	71 ± 69 (1) ^{A}
Scots pine	142 ± 38 $^{BC\,a}$	118 ± 23 $^{A\,a}$	101 ± 27 $^{A\,a}$	21 ± 13 ^{A}
Douglas fir	117 ± 69 $^{AB\,a}$	93 ± 34 $^{A\,a}$	98 ± 37 $^{A\,a}$	25 ± 10 ^{A}
		Cd ($CaCl_2$)		
silver birch	0.56 ± 0.21 $^{A\,a}$	0.68 ± 0.31 $^{A\,a}$	0.60 ± 0.33 $^{A\,a}$	0.26 ± 0.20 ^{A}
oak	0.67 ± 0.22 $^{A\,a}$	0.81 ± 0.24 $^{A\,a}$	0.65 ± 0.26 $^{A\,a}$	0.13 ± 0.13 ^{A}
black locust	0.58 ± 0.18 $^{A\,a}$	0.64 ± 0.40 $^{A\,a}$	0.52 ± 0.31 $^{A\,a}$	0.16 ± 0.10 ^{A}
aspen	0.45 ± 0.15 $^{A\,a}$	0.52 ± 0.21 $^{A\,a}$	0.49 ± 0.22 $^{A\,a}$	0.31 ± 0.20 ^{A}
Scots pine	0.87 ± 0.50 $^{A\,a}$	0.72 ± 0.38 $^{A\,a}$	0.56 ± 0.43 $^{A\,a}$	0.16 ± 0.13 ^{A}

Douglas fir	0.64 ± 0.15 $^{A\,a}$	0.57 ± 0.14 $^{A\,a}$	0.53 ± 0.16 $^{A\,a}$	0.14 ± 0.11 ^{A}
		Zn ($CaCl_2$)		
silver birch	32 ± 11 $^{A\,a}$	28 ± 14 $^{A\,a}$	27 ± 13 $^{A\,a}$	11 ± 8 ^{A}
oak	38 ± 11 $^{A\,ab}$	41 ± 19 $^{A\,b}$	35 ± 19 $^{A\,a}$	8 ± 8 ^{A}
black locust	30 ± 9 $^{A\,a}$	34 ± 20 $^{A\,a}$	30 ± 19 $^{A\,a}$	6 ± 5 ^{A}
aspen	33 ± 17 $^{A\,a}$	26 ± 13 $^{A\,a}$	22 ± 11 $^{A\,a}$	14 ± 11 ^{A}
Scots pine	43 ± 19 $^{A\,a}$	43 ± 34 $^{A\,a}$	28 ± 17 $^{A\,a}$	8 ± 7 ^{A}
Douglas fir	36 ± 15 $^{A\,a}$	26 ± 5 $^{A\,a}$	26 ± 9 $^{A\,a}$	4 ± 2 ^{A}
		Cd (NH_4OAc-EDTA)		
silver birch	1.34 ± 0.77 $^{A\,a}$	1.29 ± 0.76 $^{A\,a}$	1.67 ± 1.12 $^{A\,a}$	0.39 ± 0.37 ^{A}
oak	1.22 ± 0.47 $^{A\,a}$	1.76 ± 0.88 $^{A\,b}$	1.67 ± 0.95 $^{A\,ab}$	0.39 ± 0.71 ^{A}
black locust	1.30 ± 0.64 $^{A\,a}$	1.72 ± 0.96 $^{A\,a}$	1.41 ± 0.87 $^{A\,a}$	0.27 ± 0.26 ^{A}
aspen	3.26 ± 1.18 $^{B\,b}$	1.91 ± 1.10 $^{A\,a}$	1.78 ± 0.96 $^{A\,a}$	1.08 ± 0.93 ^{A}
Scots pine	1.96 ± 0.83 $^{AB\,a}$	2.09 ± 0.36 $^{A\,a}$	1.80 ± 1.11 $^{A\,a}$	0.29 ± 0.29 ^{A}
Douglas fir	1.84 ± 0.21 $^{AB\,a}$	2.10 ± 0.97 $^{A\,a}$	2.31 ± 0.96 $^{A\,a}$	0.25 ± 0.24 ^{A}
		Zn (NH_4OAc-EDTA)		
silver birch	61 ± 35 $^{A\,a}$	43 ± 28 $^{A\,a}$	55 ± 32 $^{A\,a}$	15 ± 11 ^{A}
oak	58 ± 24 $^{A\,a}$	71 ± 48 $^{AB\,a}$	71 ± 51 $^{A\,a}$	20 ± 29 ^{A}
black locust	59 ± 31 $^{A\,a}$	72 ± 35 $^{AB\,a}$	63 ± 32 $^{A\,a}$	12 ± 10 ^{A}
aspen	200 ± 49 $^{B\,b}$	74 ± 47 $^{AB\,a}$	70 ± 47 $^{A\,a}$	48 ± 46 ^{A}
Scots pine	83 ± 28 $^{A\,a}$	94 ± 47 $^{B\,a}$	70 ± 23 $^{A\,a}$	15 ± 16 ^{A}
Douglas fir	77 ± 21 $^{A\,a}$	71 ± 27 $^{AB\,a}$	82 ± 33 $^{A\,a}$	8 ± 3 ^{A}

(1), (2), (3), (4) the number between brackets is the number of values lower than the determination limit of 0.40 for Cd or 5.0 for Zn. The mean was calculated by equalling those values to the half of the determination limit.

Correlation between soil characteristics and metal concentrations

Significant ($p < 0.01$) positive correlations were found between pH, CEC, OC and the total Cd and Zn concentrations in the topsoil (Fig 3.3). The three considered soil characteristics appeared to affect total metal concentrations significantly. The correlation coefficients with the $CaCl_2$-extractable metal concentrations were all negative but much weaker than those with the total concentrations. Only the influence of pH on extractable Cd was significant.

3.4 Discussion

The Cd and Zn concentrations in the tree compartments and the Cd and Zn redistribution in the different soil layers are the net result of a variety of biogeochemical and biophysical processes that took place during 10 years after afforestation. The most apparent tree species effects on the metal redistribution and the consequent implications for phytostabilization will be discussed.

3.4.1 Tree species effects on above-ground metal accumulation

Poplars (*Populus* spp.) and *Salix* spp. are known to take up Cd and Zn from polluted soils and to accumulate these metals in their biomass, including branches, leaves and wood, distinctly more than other tree species (Vandecasteele et al., 2003; Brekken and Steinnes, 2004; Laureysens et al., 2004; Mertens et al., 2007; Unterbrunner et al., 2007; Hassinen et al., 2009). The Cd and Zn concentrations measured in the biomass of aspen (*Populus tremula*) are in line with these reports. Our findings also confirm that birches (*Betula* spp.) do not take up Cd but are known as Zn accumulators (Alriksson and Eriksson, 2001; Rosselli et al., 2003). Furthermore, a clear compartmentalization between the three studied biomass fractions was observed, in particular for Zn. Tree species that take up Cd or Zn from the rooting zone obviously translocate those trace metals to their leaves, as reported before (Alriksson and Eriksson, 2001; Hammer et al., 2003; Vervaeke et al., 2003; Meers et al., 2005; Mertens et al., 2007; Unterbrunner et al., 2007).

Above-ground metal amounts (Table 3.4) represent the actual metal stock in the above-ground biomass and can be used to estimate metal fluxes by litterfall and wood immobilization. Considering the risk of metal dispersion within ecosystems, litterfall should be given principal attention as it brings the total foliar Cd and Zn stock to the upper soil layer every autumn, or continuously in case of the conifer species. The high Cd and Zn stocks in the leaf litter of aspen represent large Cd and Zn fluxes every autumn and generate consequently a pathway of above-ground metal dispersion. On the other hand, the large Cd and Zn stocks in aspen's woody biomass will have smaller implications with regard to ecotoxicological risks, since the woody compartment more slowly enters the decomposition cycle.

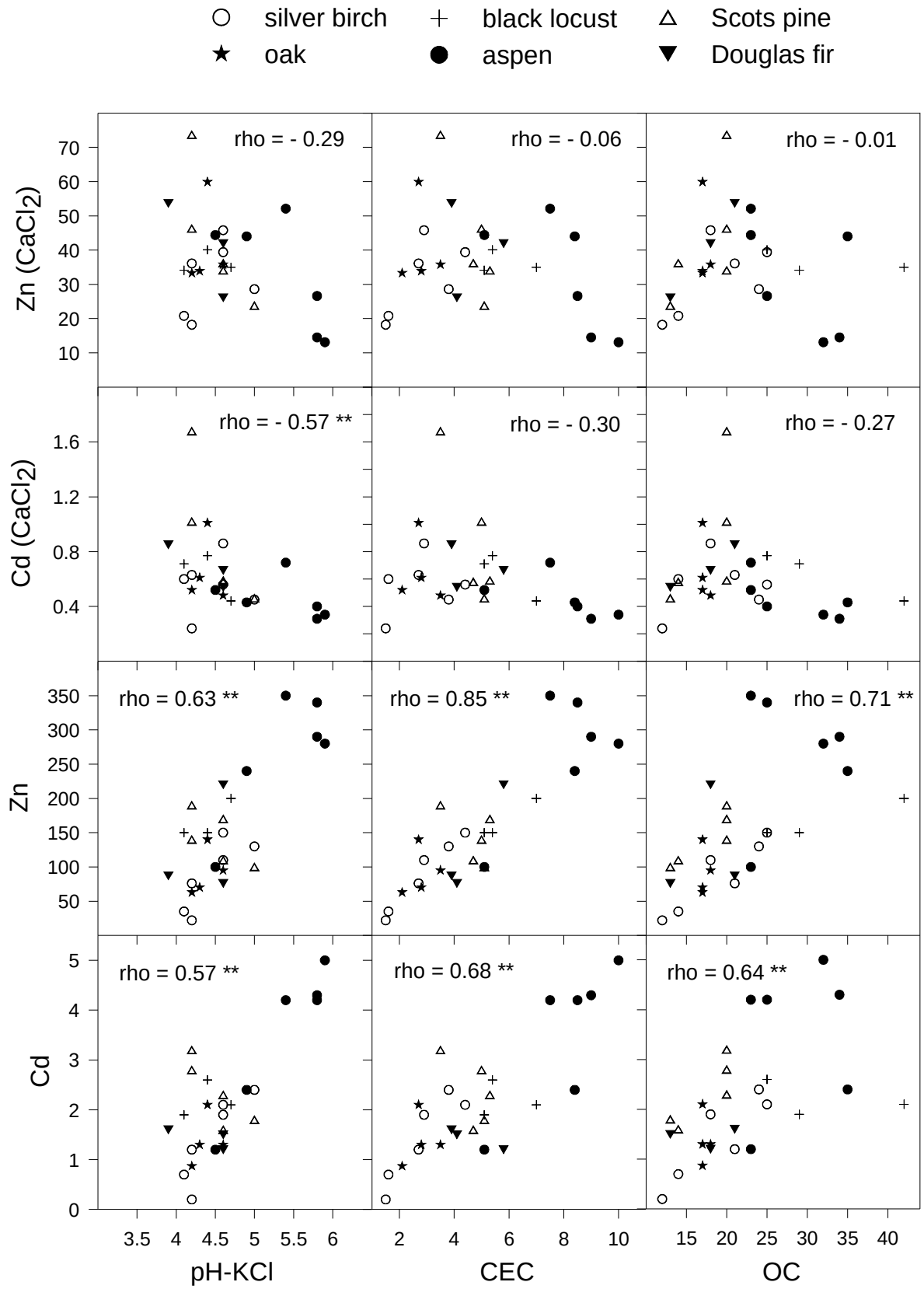

Figure 3.3 Correlations between the soil characteristics pH (-), CEC (cmol kg^{-1}), OC (g kg^{-1}) and the total and $CaCl_2$-extractable Cd and Zn concentrations (mg kg^{-1} DM) in the topsoil (0-5 cm) under the six tree species, with Spearman's rho correlation coefficients ($p < 0.01$)**

3.4.2 Tree species effects on soil pH, OC and CEC

It is generally known that plant species can differ in their effect on soil pH, organic matter content and CEC, and differences between tree species for these soil characteristics have been reported in the past (Nordén, 1994; Finzi et al., 1998; Augusto et al., 2002; Hagen-Thorn et al., 2004; Reich et al., 2005; Mertens et al., 2007). Trees are known to lower the soil pH through a variety of processes such as soil respiration, nutrient uptake and litter decomposition (Nilsson et al., 1982; Reich et al., 2005; De Schrijver et al., 2012). In the present study, however, aspen significantly increased the pH in its topsoil (0-5 cm) compared to the deeper soil layer and to the other tree species (Table 3.5). No significant pH changes were found (yet) under the other species. The fact that aspen increased the topsoil pH was thus remarkable, particularly given the short period of tree growth (10 years) and the nutrient poor sandy soil conditions. Sandy soils are characterized by a low CEC and a low acid neutralizing capacity, which makes them more vulnerable to acidification. The differences in topsoil pH between the species can probably be attributed to the chemical properties and the decomposition rate of the different leaf litter types (Hagen-Thorn et al., 2004; Reich et al., 2005; Mertens et al., 2007). Slow litter decomposition leads to the production of organic acids and delays the return of base cations to the soil, which will result in lower pH values (Hagen-Thorn et al., 2004). Leaf litter from poplars is generally of high nutritional quality, facilitating rapid litter decomposition and nutrient release to soils (Cooke and Weih, 2005). In a common garden test with 14 tree species Reich et al. (2005) found that litter Ca concentration appeared to be a key driver of changes in soil properties and that soil pH increased with the Ca concentration of foliage litter. Our results showed that aspen indeed had the highest Ca concentrations in its leaf litter (unreported data). The ameliorating effect of the nutrient rich aspen litter on pH did not occur yet in the deeper soil layers. Black locust is a nitrogen (N)-fixing legume tree (Boring and Swank, 1984) and litter decomposition rates of N-fixing species are reported to be higher than those of non-N-fixing species (Aerts and Chapin, 2000). However, no increase of the topsoil pH under black locust was found, most likely because symbiotic N_2-fixation can lead to soil acidification as a by-product of increased nitrification rates (Van Miegroet and Cole, 1985; Johnson and Lindberg, 1992; Rhoades et al., 2001).

As CEC is influenced by the clay and organic matter content and the soil pH (Helling et al., 1964), differences in CEC at this sandy site will mainly be determined by variations in organic matter content. The CEC was indeed strongly positively correlated with OC in each soil layer. Due to input of organic material from litterfall, trees increase the amount of organic matter in the upper soil layers which will eventually lead to the formation of an ectorganic horizon (Emmer, 1995). No ectorganic horizon was discernible yet under this young forest. Nevertheless, the OC

contents in the topsoil under aspen and black locust, i.e. those species with high litter decomposition rates, could be distinguished from the other species. This was also reflected in a higher CEC under aspen.

The values of the three examined soil characteristics in the deepest layer (50-60 cm) were significantly lower than at 20-30 cm depth. This could be attributed to the former agricultural use of the site, which aimed at maintaining a high pH and organic matter content in the plough layer by liming and fertilizing.

Effects of the different tree species on the soil characteristics after only 10 years of tree growth on a poor sandy soil were remarkable and important, considering the strong influence of pH, CEC and OC on metal mobility, as shown in Fig 3.3. Both the positive and negative relations between the soil characteristics and metal concentrations were expected and confirmed previous findings (Römkens and Salomons, 1998; Sauvé et al., 2000a; Andersen et al., 2002; Watmough et al., 2005).

3.4.3 Tree species effects on metal redistribution in the soil

Input and output fluxes determine metal concentrations and metal amounts in the different soil layers. Output of metals from the soil layers is mainly controlled by uptake by plant roots and leaching. Input to the upper soil layer is determined by input of contaminated litter and atmospheric deposition. Input via atmospheric deposition was not accounted for in this study, since the metal emissions from the factory nearby decreased significantly before the site was afforested. However, secondary deposition due to resuspension of contaminated soil may nowadays contribute to metal input to the topsoil. See § 5.4.2 for further reflections on this issue.

The most distinct species effect on metal redistribution was found for aspen, showing higher total Cd and Zn concentrations in the topsoil compared to the other species. Total Cd and Zn concentrations in the upper 5 cm were on average respectively 1.6 and 2 times higher than those at 20-30 cm depth and respectively 2 and 2.5 times higher than under the other species. Such a metal redistribution can be attributed to an accumulation in the topsoil or to leaching of metals from the deeper layers. Whether accumulation of metals via the litterfall causes an increase of the soil concentration depends on two conditions: (1) the concentration in the decomposed litter is higher than the soil concentration and (2) the input flux is greater than the output flux. Decomposition of litter slows down and stops at a 'limit value' (fraction of litter that does not decompose) of 0-55% of the dry weight (Berg, 2000). As metals are immobilized by humic substances during litter decay (Stevenson, 1982; Laskowski and Berg, 1993), metal concentrations

in the litter will at least double during decomposition from fresh leaves to decomposed litter. The Cd and Zn concentrations of the decomposed litter of the aspens thus are expected to increase to about 26 mg Cd kg^{-1} and 4700 mg Zn kg^{-1} dry weight (Table 3.3). They will therefore be significantly higher than the soil concentrations, and allow an increase of the topsoil concentrations to occur. However, this only holds true if output fluxes of metals do not exceed the input flux. In addition, the relatively high values for pH, OC and CEC found in the topsoil under aspen contribute to the adsorption of the metals on the soil's exchange sites (Sauvé et al., 2000a). This was reflected in the fact that (1) the $CaCl_2$-extractable Cd and Zn concentrations in the topsoil were not increased under aspen and (2) neither the total Cd and Zn concentrations in the 10-20 cm layer were increased compared to 20-30 cm depth (Table 3.6). Thus, the input flux of metals via the litterfall exceeded the output flux, resulting in a Cd and Zn accumulation in the topsoil under aspen. A similar accumulation of Cd and Zn in the topsoil of *Populus*, compared to other species, was reported on a polluted dredged sediment disposal site with 33-year-old trees (Mertens et al., 2007). Nevertheless, our results were striking as the trees were only 10 years old and sandy soils show faster dynamics in metal mobilization than calcareous dredged sediments.

In contrast to aspen, the high Zn concentration in the leaf litter of silver birch (Table 3.3) was not reflected in a considerable Zn accumulation in the topsoil compared to the 20-30 cm layer (Table 3.6). This can probably be attributed to the low leaf litter biomass production of silver birch (Table 3.2). The other tree species did not show any marked Cd or Zn accumulation in their above-ground biomass and consequently no metal accumulation in the topsoil could be observed. On the contrary, for some species we observed a slight decrease in the total topsoil concentrations compared to the deeper layers, although this trend was not significant. This effect was most pronounced for oak (Table 3.6). Leaching of the metals to deeper soil layers, due to soil acidification, might be one of the reasons for that decrease.

The total Cd and Zn concentrations in the upper three soil layers were compared with the background concentrations and with the Flemish soil sanitation reference values (Vlarebo, 2008). These values are assessed as a function of the organic matter (2.71%) and clay contents (3.33%) of the soil (Table 3.7). In more than half of the sampling points, total Cd concentrations in the upper three soil layers exceeded the soil sanitation reference value, whereas for Zn this limit was not exceeded at the study site. Total and extractable Cd and Zn concentrations in the deepest soil layer (50-60 cm) were significantly lower than at 20-30 cm depth (Table 3.6). This could be explained because the soil contamination at our study site was mainly caused by atmospheric pollution. Ploughing till approximately 40 cm depth redistributed the metals in the upper three sampling layers, but not deeper.

Table 3.7 Background concentration and soil sanitation norm for Cd and Zn in Waaltjesbos, taking into account %OM and %clay (Vlarebo, 2008)

	Cd ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)
background concentration	0.64	56
soil sanitation reference value (type I)	1.59	544

Unlike total soil metal concentrations, extractable metal concentrations allow an estimation of the mobile metal pools in the soil, thus reflecting a metal fraction that could potentially be taken up by adjacent plant roots, be detrimental to various soil biological organisms, or otherwise be leached from the soil and contaminate groundwater and surface water (Sauvé et al., 2000a; Weng et al., 2001a). Extractable metal concentrations thus offer a better criterion when it comes to evaluation of possible dispersion risks of the metals in the environment or in the food chain. Our results of the $CaCl_2$-extractable Cd and Zn concentrations in the soil profile showed no significant tree species effects (Table 3.6). It was, moreover, particularly remarkable that aspen did not generate elevated $CaCl_2$-extractable concentrations in the topsoil. This could be explained by the higher pH and CEC under aspen (Table 3.5, Fig 3.3). This implies that the risks concerning aspen might actually be smaller than expected from the accumulation of total Cd and Zn in the topsoil. However, NH_4OAc-EDTA-extractable Cd and Zn concentrations in the topsoil under aspen were elevated compared to other species and to deeper soil layers. Being a very strong extractant, NH_4OAc-EDTA yielded almost 'totals' on this poorly buffered sandy soil. The implications of these findings with respect to risk control in phytostabilization projects are discussed below (see § 3.4.4).

3.4.4 Implications for phytostabilization

Phytostabilization uses plants to minimize the mobility and bioavailability of pollutants in the environment, either by stabilizing them or by preventing their migration (Smith and Bradshaw, 1972; Vangronsveld et al., 1995b). Consequently, tree species that accumulate metals in their leaves and that induce soil acidification should be avoided, as these processes might give rise to dispersion of metals in the environment via above- and below-ground pathways.

In Chapter 2 (see § 2.4), we identified three main risks associated with the accumulation of metals in above-ground plant parts: (i) metals entering the food chain through herbivores, (ii) dispersion of contaminated plant material to adjacent environments and (iii) accumulation of metals in the

topsoil. The topsoil is particularly vulnerable as it is the biologically most active part of the soil system and biological activity has been shown to be highly sensitive to metal pollution (Bergkvist et al., 1989). In this respect, aspen and *Populus* species in general should be avoided for afforesting Cd and Zn contaminated lands because they translocate high amounts of Cd and Zn into the foliage. Our results clearly demonstrate an accumulation of total Cd and Zn concentrations in the topsoil under aspen trees and this only after 10 years of tree growth. This accumulation pattern is expected to be continued in the future. Such a metal redistribution in the ecosystem is undesirable in phytostabilization projects. However, 'bioavailable' $CaCl_2$-extractable Cd and Zn concentrations appeared not to be elevated under aspen, due to the higher pH and CEC values in the topsoil of this species. As extractable 'bioavailable' concentrations should preferably be considered for risk analysis, one may argue that on the short term there are few concerns regarding the ecotoxicological risks linked with aspen. However, the NH_4OAc-EDTA-extractable Cd and Zn concentrations in the topsoil under aspen were increased compared to the deeper soil layers, as was also reported by Mertens et al. (2007) for *Populus* 'Robusta'. This indicates that in more acid conditions the accumulated Cd and Zn in the topsoil will yet become more mobile and thus 'bioavailable'. This could for instance be the case when the aspen trees would be harvested and replaced by more acidifying species. Moreover, it should be stressed that all metals accumulated in the leaves might still pose a long-term risk to primary consumers and enter the food chain.

Although silver birch translocated considerable amounts of Zn to its leaves, its low leaf litter biomass production mitigated the risk of Zn dispersion in the ecosystem, as confirmed by the absence of Zn accumulation in the topsoil under birch. Above-ground dispersion of Zn from silver birches might, however, still occur via herbivory. Furthermore, as leaf litter biomass production will increase in time, Zn accumulation in the topsoil can potentially occur in the future.

Soil acidification might give rise to metal leaching to deeper soil layers and eventually to groundwater contamination. It was shown that metal solubility increases rapidly when solution pH drops below a critical value. Cd and Zn leaching breakthrough was found to occur within the solution pH range of 4.0 to 4.5 (Bergkvist et al., 1989). pH-H_2O values at our site were > 5.3 in all soil layers [deduced from pH-KCl (Table 3.5) according to the formula pH-H_2O = 0.967 pH-KCl + 1.127 (Azevedo et al., 2013)], implying that the Cd and Zn leaching breakthrough point was not attained yet. However, continued tree growth in the future will most likely result in a more progressive acidification of the upper soil layers. Given the risk of Cd and Zn leaching, we recommend not to plant acidifying tree species, or else to mix them with other species that have

high nutritional leaf litter quality, facilitating rapid litter decomposition and nutrient release to soils, e.g. European rowan (*Sorbus aucuparia*) or alder buckthorn (*Rhamnus frangula*). More research on the effects of these shrub species is done in Chapter 6.

The effects of the different tree species on the soil characteristics and on the metal redistribution in the soil profile will evolve during the next decades and will probably become more pronounced in the future. A next sampling campaign within 10 or 20 years is therefore essential.

Finally, based on the present study, it was not clear which tree species may be planted on Cd and Zn contaminated sandy soils, without risks for above- and below-ground metal dispersion. The next chapters of this thesis will contribute to answering this question.

4

Metal and nutrient dynamics in decomposing tree litter on a metal contaminated site

Abstract

In a forest on sandy, metal polluted soil, we examined effects of six tree species on litter decomposition rates and accompanied changes in metal (Cd, Zn) and nutrient (base cations, N, C) amounts. Decomposition dynamics were studied by means of a litterbag experiment lasting for 30 months. The decomposition peak occurred within the first year for all tree species, except for aspen. During litter decomposition, high metal litter types released part of their accumulated metals, whereas low metal litter types were characterized by a metal enrichment. Base cations, N and C were released from all litter types. Metal release from contaminated litter might involve risks for metal dispersion towards the soil. On the other hand, metal enrichment of uncontaminated litter may be ecologically relevant as it can be easily transported or serve as food source.

After: Van Nevel, L., Mertens, J., Demey, A., De Schrijver, A., De Neve, S., Tack, F.M.G., Verheyen, K., 2014. Metal and nutrient dynamics in decomposing tree litter on a metal contaminated site. Environmental Pollution, 189, 54-62.

4.1 Introduction

Decomposition dynamics of plant litter has been studied in a plethora of studies during the last decades (Prescott, 2005, 2010), reflecting the importance of litter decomposition in the recycling of elements within terrestrial ecosystems (Meentemeyer, 1978; Melillo et al., 1982; Blair, 1988a, 1988b; Vitousek et al., 1994). Litter decomposition rates also determine soil acidification and dissolved organic carbon (DOC) leaching (Konova, 1966; Finzi et al., 1998; Fröberg, 2004; Hagen-Thorn et al., 2004). Both processes are essential on metal contaminated sites as soil acidification and DOC production are likely to affect metal mobilization and, subsequently, metal leaching to deeper soil layers (Mayer, 1998; Strobel et al., 2001a).

It has long been demonstrated that litter decomposition rates are controlled by environmental conditions, by the chemical composition of the litter and by soil organisms (e.g. Meentemeyer, 1978; Swift et al., 1979; Melillo et al., 1982; Coûteaux et al., 1995; Gholz et al., 2000; Hättenschwiler and Gasser, 2005; Cornwell et al., 2008; Wickings et al., 2012). In general, climate (especially temperature and moisture) governs decay rates on a broad regional scale, whereas initial litter quality (C:N ratio, lignin, N, lignin:N ratio, base cation and polyphenol content) are of more importance in controlling decay rates at a smaller scale, e.g. within site (Berg et al., 1993; Coûteaux et al., 1995; Heal et al., 1997; Aerts and Chapin, 2000). However, other authors (Cornwell et al., 2008; Zhang et al., 2008) revealed, on the basis of meta-analyses, that litter quality was the dominant regulator of litter decomposition at the global scale.

Another important aspect of litter decomposition, besides the decomposition rates, are the related enrichment and release dynamics of chemical elements, as these reflect elemental cycling and availability to plants and soil organisms. Although it has been well documented that accumulated trace metals can retard litter decomposition processes (e.g. Tyler, 1976; Coughtrey et al., 1979; Berg et al., 1991; Laskowski et al., 1994; McEnroe and Helmisaari, 2001; Johnson and Hale, 2004), knowledge of the dynamics of metals in decomposing litter is relatively scarce (Virzo De Santo et al., 2002; Lomander, 2002). Especially field studies exploring metal dynamics in decomposing litter on metal contaminated sites are rare. Most of the studies were executed in unpolluted forest ecosystems (Laskowski and Berg, 1993; Laskowski et al., 1995; Lomander and Johansson, 2001; Virzo De Santo et al., 2002; Tyler, 2005; Kaila et al., 2012). We found only one study where dynamics of metal fluxes in decomposing leaves were determined on polluted soil (Scheid et al., 2009).

In a 14-year-old, metal contaminated forest containing replicated stands of six different tree species, we studied, by means of a litterbag experiment, (i) litter decomposition rates for different

tree species, (ii) the accompanied changes in metal and nutrient amounts and (iii) the relations between metal and nutrient dynamics, forest floor leachate fluxes and topsoil characteristics. We hypothesize that (i) tree species with low litter quality (in terms of base cations and C/N ratio) and/or high Cd and Zn litter concentrations have slower litter decomposition rates and (ii) metal and nutrient release from decomposing litter will be reflected in topsoil characteristics and forest floor leachate fluxes. No hypotheses were made on the extent and direction of the metal dynamics (enrichment or release) during decomposition of the different litter types, due to scarce knowledge on this topic.

4.2 Materials and Methods

4.2.1 Experimental set-up and sampling

The study included the six considered tree species (oak, silver birch, black locust, aspen, Scots pine, Douglas fir) and was executed in the selected stands in 'Waaltjesbos' (see Fig 3.1). See § 3.2.1 for full site descriptions and stand selection. A characterization of the soil of the study site is given in Table 3.5 (soil characteristics pH-KCl, OC content, CEC) and Table 3.6 (total and extractable soil Cd and Zn concentrations). The six tree species are characterized by divergent leaf litter quality and different metal concentrations in their leaf litter (see Tables 4.1 and 5.1).

Freshly fallen leaves and needles were collected randomly from the forest floor in the considered stands in November 2009 and air-dried at 25 °C in a forced air oven. Air-dried foliar litter (20 g) was put in 30 x 30 cm litterbags with a mesh size of 1.5 x 1.5 mm. For Douglas fir, we used litterbags with a smaller mesh size (1 x 0.6 mm) because of needle loss through the bigger meshes. Macrofauna was excluded from both litterbag types (Herlitzius, 1983; Staaf, 1987; Smith and Bradford, 2003; Hättenschwiler et al., 2011) and therefore the decomposition process was mainly mediated by microflora and micro- and mesofauna. Hence, we are confident that decomposition dynamics of Douglas fir litter elapsed under comparable circumstances as that of the other species. Pooled samples (1 per tree species) of the collected foliar litter were also used for determination of initial litter quality (see § 4.2.2 for details on chemical analyses).

In February 2010, series of 12 litterbags were put in every stand - each species in its respective stand - on top of the mineral soil. Three replicate litterbags per species (1 per stand) were collected after 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24 and 30 months. For black locust and aspen, known to produce easily decomposable litter (Aerts and Chapin, 2000; Cooke and Weih, 2005;

Tateno et al., 2007), only 10 litterbags were placed in every stand, the last being collected after 20 months. Hence, we sampled 30/36 litterbags per species during the course of the experiment.

Since aspen and silver birch are the only species that showed elevated Cd and/or Zn concentrations in their leaf litter (see Table 3.3), we consider those two species here as 'high metal litter types' and the other *in situ* species as 'low metal litter types'. Additionally, litterbags with *ex situ* uncontaminated leaf litter from aspen and silver birch were brought to the study site as well, and placed in the aspen and silver birch stands respectively. In that way, we were able to observe decomposition dynamics in 'high metal litter types' (aspen Cd and Zn; birch only Zn) as well as in 'low metal litter types' (non-accumulating *in situ* species and uncontaminated aspen and birch). However, comparison of contaminated versus uncontaminated aspen and birch litter was not at issue, since our experimental set-up could not account for the distinction between 'contamination effects' and 'site effects' (manifested by different nutrient levels in the litter, see Table 4.1) of the two litter types.

Uncontaminated leaf litter was collected from the forest floor in two other local forests: 'Heidebos' (51°10'55" N, 3°54'25" E) and 'Dombergheide' (51°21'3" N, 4°57'10" E), for silver birch and aspen, respectively. Both forests are also located on poor sandy soils, but without a history of metal pollution. At the moment of leaf litter collection, the birch and aspen stands were about 50 and 20 years old, respectively. The pretreatment and sampling of the uncontaminated litterbags was identical to that of the *in situ* litterbags, i.e. 12 collections for uncontaminated silver birch and 10 collections for uncontaminated aspen.

To allow extrapolation of the changes in absolute nutrient and metal amounts during decomposition (see further) from litterbag scale to stand scale, we estimated leaf litterfall amounts for each tree species. Although we had determined annual leaf litterfall already in 2007-2008 (see Table 3.2), we measured it again as we expected substantial tree growth given the young age of the forest. Leaf litterfall was again collected with litterfall traps that had a circular surface area of 0.24 m^2. For each tree species, one stand was allocated for leaf litterfall estimation and provided with six litterfall traps (end of August 2012) which were emptied monthly during autumn 2012 until the broadleaved trees were leafless. Litter samples were oven-dried (70 °C) to constant weight. Prior to weighing (± 0.1 g), samples were sorted out by hand to separate the leaf litter of the considered tree species while discarding other litterfall fractions. As coniferous litter is shed year-round, annual litterfall amounts of the coniferous species were obtained by upscaling their litterfall amounts gained during autumn 2012. Therefore we used conversion factors based on the annual needle litterfall measured in 2007-2008.

In order to compare leaf litter decomposition in litterbags with forest floor dynamics, the forest floor mass was determined. Forest floor was defined as the organic material above the mineral soil (i.e. Ol + Of horizons as no Oh horizon was discernible yet under this young forest; Of horizon had not been developed yet in the black locust and Douglas fir stands) (Jabiol et al., 1995). We collected forest floor samples in March 2012 using a 20 x 20 cm wooden frame (6 replicates per stand, thus 18 replicates per tree species). Forest floor samples were oven-dried (70 °C) to constant weight, fruits and non-litter material were discarded and the remaining litter fraction was weighed (± 0.01 g).

Finally, we related metal and nutrient dynamics in the decomposing litter to forest floor leachate fluxes and topsoil characteristics. Forest floor leachate was collected in 2009-2010 using zero-tension lysimeters, installed directly under the forest floor in the same stands as those where the litterbags were put (see Chapter 5). Topsoil (0-5 cm) was sampled in 2007, also in the same stands (see Chapter 3).

4.2.2 Sample analysis

Upon collection, litterbags were air-dried at 25 °C in a forced air oven. Thereafter, prior to weighing (± 0.1 g), litter samples were carefully cleaned by hand from extraneous material. Subsequently, prior to chemical analysis, litter was oven-dried (70 °C) to constant weight and ground using a centrifugal mill (Retsch ZM1, Germany). Ground samples were analyzed for Cd, Zn, Ca, K, Mg, Na, C and N. The samples were ashed at 450 °C and dissolved in HNO_3 before Cd and Zn analysis using ICP-MS (Elan DRC) and ICP-OES (Varian Vista-MPX) respectively. Concentrations of K, Ca, Mg and Na were measured by means of flame atomic absorption spectrophotometry (Varian SpectrAA-240) after a destruction in HNO_3-$HClO_4$. C and N concentrations were determined by elemental analysis (Vario Max CNS, Germany). The analytical quality of the measurements was checked by including method blanks, repeated measurements of internal and certified reference samples, and by inter-laboratory tests.

Table 4.1 Initial litter concentrations of Ca, base cations (BC), N, C, Cd and Zn (n = 1)

	Ca [§] (mg kg^{-1})	BC [§] (meq kg^{-1})	N [§] (%)	C [§] (%)	C/N [§] (-)	Cd [§] (mg kg^{-1})	Zn [§] (mg kg^{-1})
silver birch	17021	1061	1.3	50.8	39.1	2.69	1749
oak	10100	739	1.4	49.5	37.1	0.38	283
black locust	21731	1359	2.2	47.6	21.6	0.89	185
aspen	21619	1519	0.9	47.4	52.7	17.1	2209
Scots pine	6665	524	1.4	49.7	35.5	1.60	309
Douglas fir	7953	570	2.0	50.0	25.0	1.22	334
uncontaminated							
silver birch	8173	567	1.5	51.2	34.1	0.51	181
aspen	12089	837	1.7	49.1	28.9	2.29	656

[§] Concentrations analyzed on pooled samples of litter collected from the forest floor in November 2009 (cfr. § 4.2.1)

4.2.3 Data analysis

The single-exponential equation $X_t = X_0 e^{-kt}$ (Olson, 1963) is widely used to fit decomposition datasets, but it is less appropriate when strong curvatures are observed in the decomposition dynamics. In that case, decomposition data often fit better to a double-exponential equation, where litter is split in a more labile and a more recalcitrant fraction (e.g. Lousier and Parkinson, 1976). However, as Rovira and Rovira (2010) argue, it is often impossible to link those fractions with quantifiable organic pools. To fit our data we therefore used their global conceptual approach, considering a single organic compartment whose decomposition rate changes with time (Rovira and Rovira, 2010). This conceptual approach can, in practice, take different forms depending on the dataset under study. We fitted our decomposition data for each tree species to three of their proposed equations: (1) exponential decrease of the decomposition rate, (2) wave-form dynamics of the decomposition rate and (3) rational-type change in the decomposition rate. See Rovira and Rovira (2010) for more detailed, mathematical descriptions of the considered equations.

In addition to the considered equations, we also fitted our dataset to the classic Olson's model, in order to show how the equations improved the fit. As there is a higher number of parameters involved in the three selected equations (see Rovira and Rovira, 2010), more uncertainty may have been added to the models, in spite of an improvement in fit (compared to Olson's model). Therefore, we compared the R^2 values of the considered models as well as $\Delta\ AIC_C = AIC_C(Eq) - AIC_C(Olson)$

$$\text{with } AIC_C = n\ log(\hat{\sigma}^2) + 2K + \frac{2K(K+1)}{n-K-1} \qquad (4.1)$$

where n is the sample size, K the number of parameters involved in the model and $\hat{\sigma}^2$ the residual variance. The best fit (highest R^2 values and lowest $\Delta\ AIC_C$ values) for all tree species was obtained for the rational-type model:

$$X_t = X_0\ e^{-\left(ct + \frac{a^3}{4b}\frac{t^4}{(t^2+b)^2}\right)} \qquad (4.2)$$

where t is time, X_0 and X_t are the initial amount of litter and the remaining amount of litter at time t, respectively, and a, b and c are model parameters. The model represents Olson's single-exponential equation, in which the decomposition rate k in itself increases initially from its lowermost value c (at $t = 0$) to its uppermost value k_{max} (at $t = \sqrt{b}$), followed by a decrease back to value c (at $t = \infty$), with $k_{max} = c + \left(\frac{a}{2\sqrt{b}}\right)^3$ (Rovira and Rovira, 2010). Such a pattern might be observed if an initial period of microbial colonization is needed before the decomposition

process starts (e.g. Wolters and Schaefer, 1993). The course of the decomposition rates k can be modeled according to:

$$k = c + \left(\frac{at}{t^2+b}\right)^3 \quad (4.3)$$

The decomposition parameters c (k_{min}), k_{max} and $\sqrt{b}$ were derived from the model output, and their confidence intervals (CI) were explored to assess tree species effects on decomposition dynamics (see Table 4.2).

To examine which litter characteristics explained litter decomposition dynamics, correlations between initial litter concentrations (base cations, N, C, Cd, Zn, C/N) and decomposition parameters on the one hand and forest floor mass on the other hand were calculated by means of Pearson correlation coefficients. As forest floor mass is determined by both litter quality and quantity, an additional correlation between forest floor mass and annual leaf litterfall amounts (see § 4.2.1) was calculated as well.

Base cation concentrations were calculated by summing the equivalents of K^+, Ca^{2+}, Mg^{2+} and Na^+. Element amounts in the litterbags were calculated by multiplying element concentrations with the remaining mass at each collection date. Changes in nutrient and metal amounts during litter decomposition were calculated both absolutely and relatively, i.e. compared to the initial amounts. To compare all considered tree species, the changes were calculated after 20 months of decomposition, because at that point the experiment was finished for black locust and aspen (see § 4.2.1). The absolute changes were extrapolated from litterbag scale to stand scale (per ha) on the basis of the estimated annual leaf litterfall amounts (see § 4.2.1). Relations between absolute changes in metal and nutrient amounts (at stand scale) and topsoil data (Cd and Zn concentrations, pH, OC content, CEC) and forest floor leachate fluxes (Cd, Zn, DOC, H^+) were explored by means of Pearson correlation coefficients. Due to different moisture levels in the litter during sample analysis (air-dried at 25 °C prior to mass determination and oven-dried at 70 °C prior to chemical analysis), the (absolute changes in) nutrient and metal amounts were slightly overestimated by 6%. However, species-specific patterns and relative differences between the species stayed unaltered.

Finally, a repeated measures ANOVA analysis was applied to leaf litter mass (M_l), with 'time' as a within-subject factor and 'species' as between-subjects factor. Differences between species were tested using a Bonferroni post hoc test. To compare all considered tree species, 10 collections (20 months) were included in the analysis, because the litterbags of black locust and aspen did not stay longer in the forest (see § 4.2.1). All statistical analyses and model fitting were performed in SPSS Statistics 21 for Windows.

Table 4.2 Estimates of the decomposition parameters k_{min}, k_{max} and $\sqrt{b}$ (average ± SE) obtained with the rational-type model (Eq. 4.2) and R^2 of the fits; differences between species were assessed by CI overlapping, values with the same letter did not differ between species ($p < 0.05$)

	k_{min} (yr^{-1})	k_{max} (yr^{-1})	$\sqrt{b}$ (yr)	R^2
silver birch	0 ± 0.075 [a]	0.603 ± 0.300 [a]	0.601 ± 0.058 [c]	0.90
oak	0.137 ± 0.048 [a]	0.514 ± 0.202 [a]	0.561 ± 0.059 [c]	0.95
black locust	0.773 ± 0.062 [b]	2.223 ± 2.669 [a]	0.055 ± 0.027 [a]	0.95
aspen	0.300 ± 0.088 [a]	0.812 ± 0.937 [a]	1.255 ± 0.510 [bc]	0.89
Scots pine	0.023 ± 0.075 [a]	1.674 ± 0.550 [a]	0.324 ± 0.023 [b]	0.94
Douglas fir	0 ± 0.076 [a]	0.912 ± 0.551 [a]	0.345 ± 0.045 [b]	0.87
uncontaminated				
silver birch	0 ± 0.122 [a]	0.722 ± 0.466 [a]	0.628 ± 0.080 [c]	0.84
aspen	0 ± 0.135 [a]	0.991 ± 0.640 [a]	0.765 ± 0.119 [c]	0.89

4.3 Results

4.3.1 Leaf litter mass loss

We compared leaf litter mass loss through the study period under the different tree species (Fig 4.1), as well as their decomposition parameters k_{min}, k_{max} and $\sqrt{b}$ (Table 4.2 & Fig 4.2) obtained by the rational-type model (Eq. 4.2). This model generally described the decomposition data well, with R^2 values ranging between 0.84 – 0.95 (Table 4.2 & Fig 4.1). After 20 months, only 20% of the initial leaf litter mass was left in the black locust litterbags. For the other species, whose litterbags stayed longer in the forest, the remaining mass ranged from 32% to 59% after 30 months (Fig 4.1). The repeated measures ANOVA and Bonferroni corrected multiple comparisons showed that average litter mass loss through the study period (20 months) was significantly faster for black locust and Scots pine compared to the other tree species, among which no differences in average mass loss were found (data not shown). Comparison of the decomposition parameters (Table 4.2) revealed that black locust exhibited the highest decomposition rate (highest k_{min}) and fastest decomposition dynamics in the initial phase (lowest $\sqrt{b}$ - time at which the decomposition rate reaches its uppermost value).

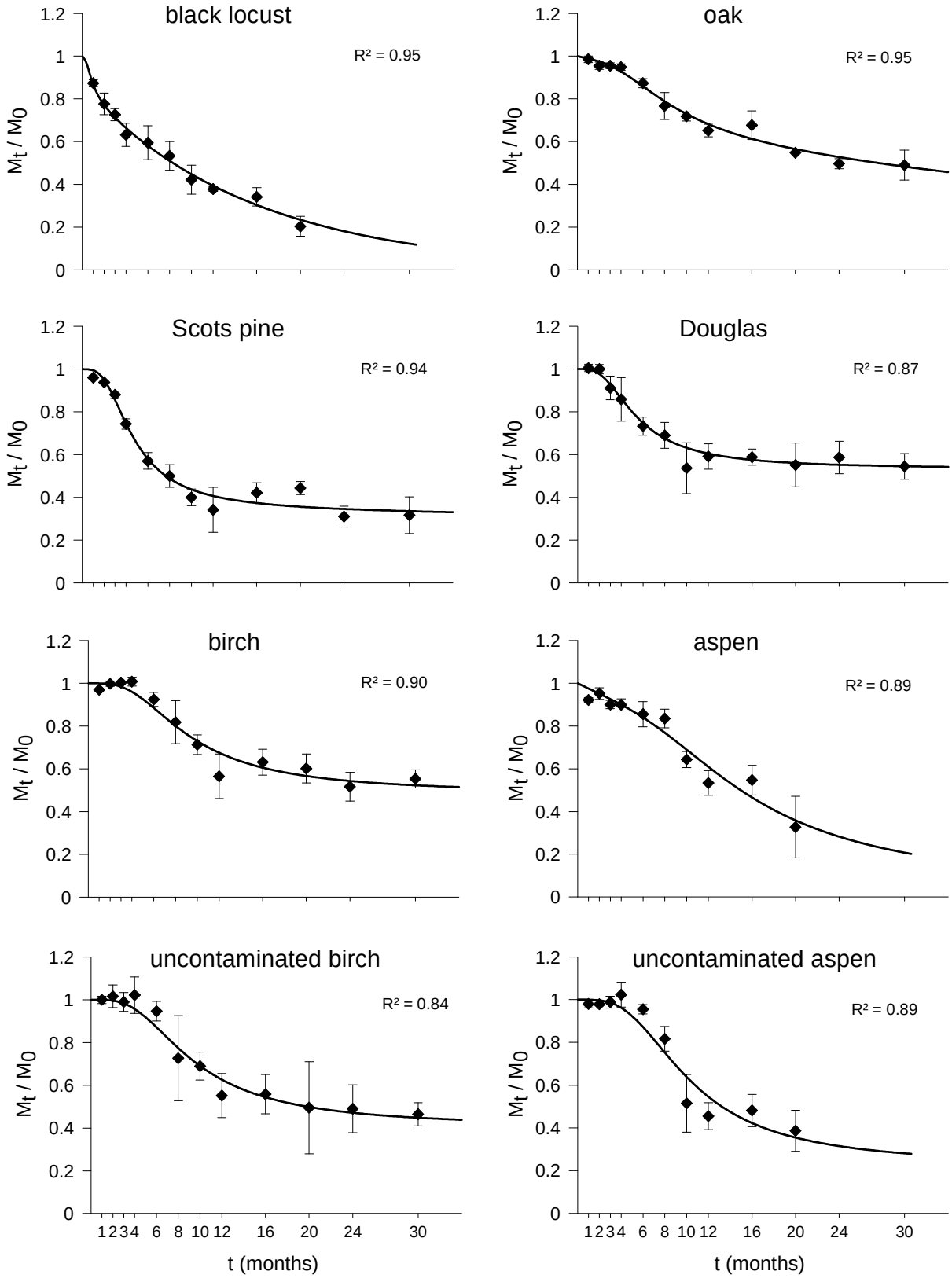

Figure 4.1 Remaining mass in the litterbags (average ± st.dev) and modeled data according to a rational-type model (Eq. 4.2; see model parameters in Table 4.2). Bonferroni corrected multiple comparisons showed that average mass loss in the first 20 months was significantly faster for black locust and Scots pine compared to the other tree species.

The dynamics of the species-specific decomposition rates *k* (Fig 4.2) revealed that the maximum decomposition rate occurred within the first year for all the tree species, except for aspen. After this decomposition peak, *k* dropped and reached almost zero after 3 years in the case of Douglas fir, Scots pine, silver birch and uncontaminated birch, whereas *k* was still relatively high after 3 years for black locust and aspen.

Based on the asymptotic curves (Fig 4.1) and the modeled *k* values that reached almost zero in the end of the modeled period (Fig 4.2), it seemed that decomposition of Scots pine, Douglas fir, silver birch and uncontaminated birch litter was strongly slowed down at the end of the study period. Litter of oak, black locust, aspen and uncontaminated aspen, on the other hand, would have decomposed more if it was left longer in the field.

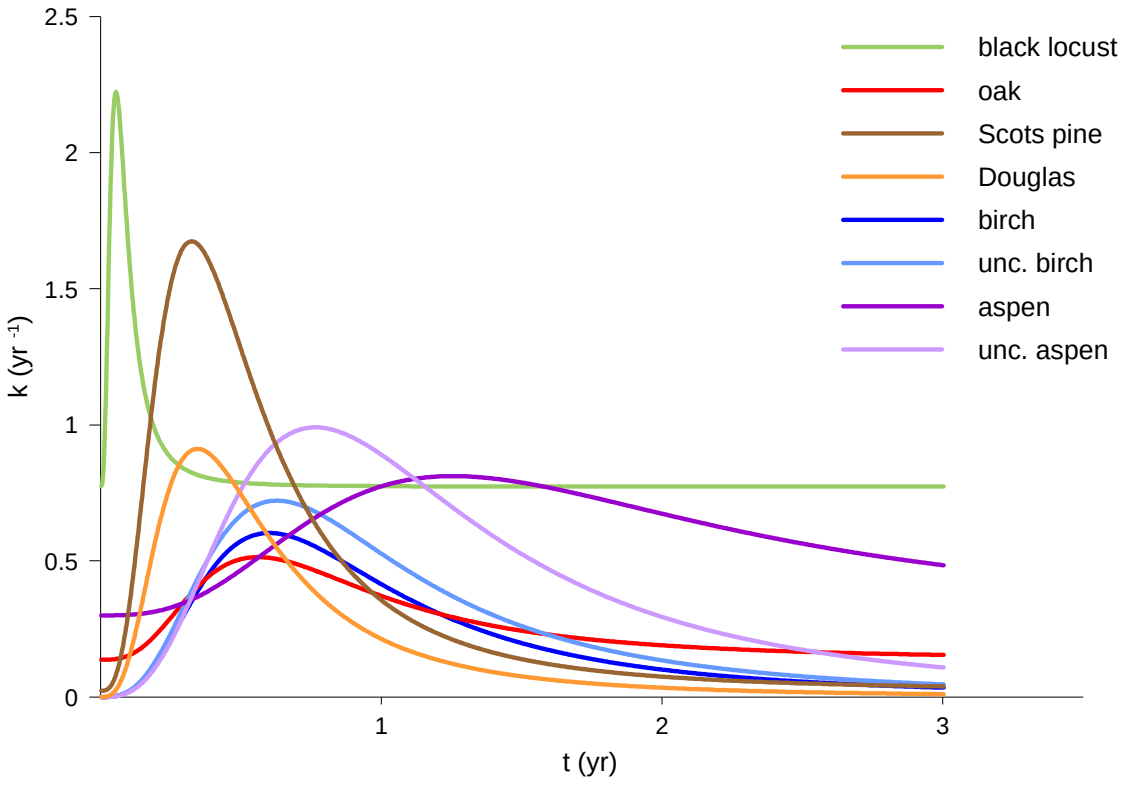

Figure 4.2 Decomposition rate (*k*) dynamics for the different tree species, modeled over 3 years according to Eq. 4.3

Correlation of the decomposition parameters with initial litter chemistry (Table 4.3) showed that mainly the timing of the decomposition peak ($\sqrt{b}$) was governed by the litter quality. Litter rich in N or with low C/N ratio had an earlier peak (low $\sqrt{b}$), whereas Cd or Zn accumulation slowed down the decomposition process (high $\sqrt{b}$). The minimum decomposition rate (k_{min}) was higher for litter that contained more base cations.

Table 4.3 Pearson correlation coefficients between initial litter concentrations (base cations (BC), N, Cd, Zn & C/N) and decomposition parameters (n = 8) and forest floor mass (n = 18)

	k_{min}	k_{max}	$\sqrt{b}$	M_{FF}
BC	0.704 *	0.242	0.344	- 0.768 **
N	0.354	0.583	- 0.773 *	- 0.146
Cd	0.169	- 0.107	0.789 *	- 0.270
Zn	- 0.034	- 0.288	0.735 *	- 0.306
C/N	- 0.205	- 0.508	0.807 **	0.009

* $p < 0.05$; ** $p < 0.01$

Forest floor mass was lowest for black locust and aspen, and highest for Scots pine (Table 4.4) and negatively correlated with initial base cation concentrations in the litter (Table 4.3). No significant correlation between forest floor mass and leaf litterfall could be found (Pearson's r = 0.291).

Table 4.4 Forest floor mass (n = 18) and annual leaf litterfall (n = 6) in Waaltjesbos (average ± st.dev); differences between species were tested using analysis of variance (ANOVA) with a Bonferroni post hoc test, values with the same letter did not differ between species (p < 0.05)

	Forest floor mass (ton DM/ha)	Annual litterfall (ton DM/ha/yr)
silver birch	9.3 ± 1.7 ^{bc}	1.4 ± 0.2 ^{a}
oak	11.4 ± 3.2 ^{bc}	3.1 ± 0.4 ^{bc}
black locust	2.4 ± 2.3 ^{a}	2.6 ± 0.2 ^{bc}
aspen	6.5 ± 1.3 ^{ab}	2.4 ± 0.3 ^{b}
Scots pine	26.2 ± 7.5 ^{d}	3.3 ± 0.2 ^{c}
Douglas fir	14.2 ± 3.6 ^{c}	7.1 ± 0.7 ^{d}

4.3.2 Metal amounts

Initial Cd and Zn concentrations were elevated in aspen litter, while silver birch litter was characterized by an elevated Zn concentration (Table 4.1). After 20 months of decomposition, aspen litter had released 50% of its initial Cd amount and 41% of its initial Zn amount (Table 4.5). There was also a slight decrease (9%) of the Zn amount in birch litter, yet this was not significant compared to the Zn dynamics in the other species. On the other hand, uncontaminated aspen and birch litter, as well as the non-accumulating *in situ* species, were characterized by an increase in metal amounts during the decomposition process (Table 4.5 & Fig 4.3). Especially oak and uncontaminated aspen and birch litter showed a considerable metal enrichment compared to their initial amounts.

4.3.3 Nutrient amounts

We found a general decrease in base cation, N and C amounts in litter of all tree species during the decomposition process (Table 4.5 & Fig 4.3). The release of base cations and C was steady, N dynamics on the other hand was more irregular (Fig 4.3). C release was evident as it is governed by the leaf litter mass loss. Base cation losses were highest from black locust and aspen litter (Table 4.5), as a result of the abundance of base cations in these litter types (Table 4.1). Net N release was highest from black locust (Table 4.5). All tree species released relatively less N than base cations and C during the decomposition process (Table 4.5). Black locust revealed highest nutrient releases, both absolutely and relatively.

Table 4.5 Absolute changes in nutrient and metal amounts in litterbags (release (-) enrichment (+)) after 20 months of litter decomposition (average ± st.dev; n = 3); relative changes (% of initial amount) between brackets; differences in absolute changes between species were tested using analysis of variance (ANOVA) with a Bonferroni post hoc test, values with the same letter did not differ between species ($p < 0.05$)

	BC (meq)	N (mg)	C (mg)	Cd (mg)	Zn (mg)
silver birch	- 8.7 ± 2.5 ^{a} (-41)	- 0.8 ± 8.3 ^{a} (-0.3)	- 4385 ± 508 ^{a} (-43)	0.03 ± 0.01 ^{a} (+53)	- 3.1 ± 3.8 ^{ab} (-9)
oak	- 6.7 ± 1.1 ^{a} (-45)	0.9 ± 27.3 ^{a} (+0.3)	- 4698 ± 144 ^{a} (-47)	0.05 ± 0.02 ^{a} (+660)	7.4 ± 2.9 ^{ab} (+131)
black locust	- 22.4 ± 0.8 ^{b} (-82)	- 318.0 ± 28.4 ^{b} (-72)	- 7727 ± 376 ^{b} (-81)	0.01 ± 0.01 ^{a} (+59)	1.8 ± 1.3 ^{ab} (+49)
aspen	- 22.2 ± 4.5 ^{b} (-73)	- 39.1 ± 66.0 ^{a} (-22)	- 6679 ± 1454 ^{ab} (-70)	- 0.17 ± 0.13 ^{b} (-50)	- 18.1 ± 21.9 ^{b} (-41)
Scots pine	- 5.7 ± 0.2 ^{a} (-55)	- 117.1 ± 11.9 ^{a} (-42)	- 5558 ± 365 ^{ab} (-56)	0.02 ± 0.01 ^{a} (+65)	3.0 ± 1.8 ^{ab} (+48)
Douglas fir	- 6.9 ± 2.0 ^{a} (-61)	- 105.7 ± 57.0 ^{a} (-26)	- 4589 ± 917 ^{a} (-46)	0.01 ± 0.01 ^{a} (+41)	0.6 ± 1.5 ^{ab} (+10)
uncontaminated					
silver birch	- 3.9 ± 3.4 ^{a} (-35)	- 89.3 ± 93.8 ^{a} (-30)	- 5696 ± 1821 ^{ab} (-56)	0.04 ± 0.01 ^{a} (+408)	23.4 ± 8.4 ^{a} (+647)
aspen	- 9.5 ± 1.7 ^{a} (-57)	- 144.9 ± 43.1 ^{a} (-43)	- 6387 ± 492 ^{ab} (-65)	0.06 ± 0.02 ^{a} (+128)	20.4 ± 5.1 ^{a} (+155)

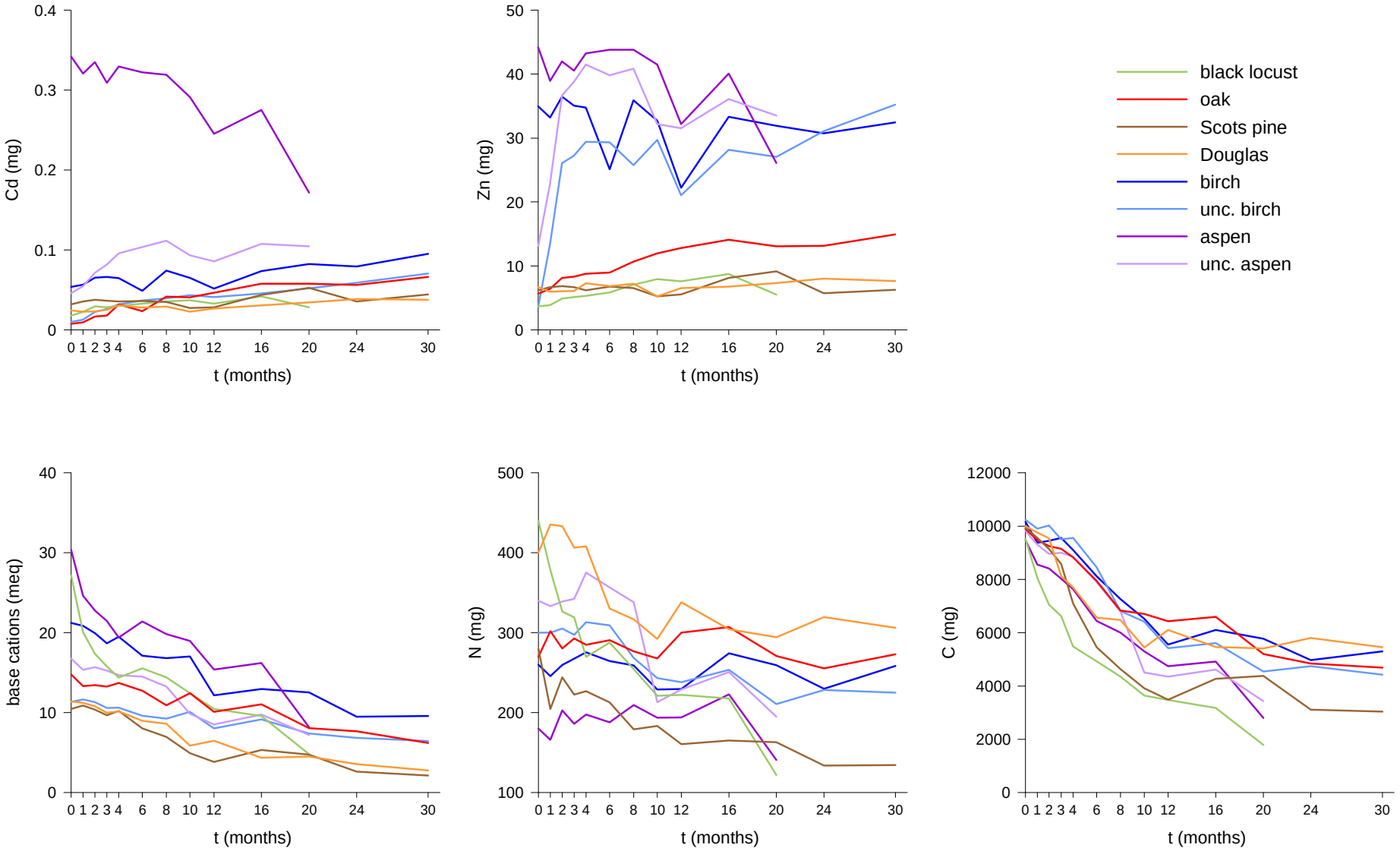

Figure 4.3 Metal and nutrient amounts (per litterbag) in decomposing litter over time under the different tree species

4.3.4 Relations with forest floor leachate fluxes & topsoil characteristics

Correlation analysis (Table 4.6) revealed that the more Cd was released from decomposing litter, the higher the Cd and Zn concentrations in the topsoil and the Cd fluxes in the forest floor leachate were. Zn release from decomposing litter was correlated with Cd and Zn topsoil concentrations but not with Zn leachate fluxes. Net release of base cations was significantly correlated with topsoil CEC and OC content (Table 4.7). No other correlations with topsoil characteristics, nor with forest floor leachate fluxes were determined.

Table 4.6 Pearson correlation coefficients between changes in metal amounts after 20 months of litter decomposition (releases/enrichments per ha) and metal forest floor leachate fluxes and topsoil (0-5 cm) concentrations (n = 36)

	$Cd_{leachate}$	$Cd_{topsoil}$	$Zn_{leachate}$	$Zn_{topsoil}$
Cd_{litter}	0.380 *	0.494 **	0.067	0.497 **
Zn_{litter}	0.236	0.369 *	- 0.063	0.349 *

* $p < 0.05$; ** $p < 0.01$

Table 4.7 Pearson correlation coefficients between changes in nutrient amounts after 20 months of litter decomposition (releases/enrichments per ha) and topsoil (0-5 cm) characteristics (pH-KCl, OC content, CEC), DOC and H+ forest floor leachate fluxes (n = 36)

	$pH_{topsoil}$	$OC_{topsoil}$	$CEC_{topsoil}$	$DOC_{leachate}$	$H^+_{leachate}$
BC_{litter}	0.243	0.562 **	0.674 **	- 0.201	- 0.237
C_{litter}	- 0.175	0.042	0.243	0.101	- 0.023
N_{litter}	- 0.292	0.316	0.239	0.056	- 0.004

* $p < 0.05$; ** $p < 0.01$

4.4 Discussion

4.4.1 Leaf litter mass loss

Our decomposition experiment allowed for the assessment of the decomposition rate peak, which occurred within the first year for all species except for aspen (Fig 4.2). This peak reflects the early decomposition stages, which are characterized by a rapidly decomposing labile pool (Adair et al., 2008). After the decomposition peak, the decay rate slowed down as the relative amount of recalcitrant material in the litterbags increased. After 30 months, the decomposition rates of Scots pine, Douglas fir, silver birch and uncontaminated birch litter had approximated zero (Fig 4.2), implying that these species had already reached their 'limit value' at the end of the study period (Fig 4.1), i.e. the extremely slow decay of the remaining recalcitrant litter fraction (Berg 2000). The decomposition of oak, black locust, aspen and uncontaminated aspen litter was still ongoing after 20/30 months (Fig 4.1), meaning that those litter types would have decomposed further if they were left longer in the field, albeit with a slower rate, inherent to the recalcitrant phase (Berg et al., 1993; Prescott et al., 2004; Prescott, 2005; Hobbie et al., 2006; Adair et al., 2008; Harmon et al., 2009).

Correlations between decomposition dynamics (expressed by k_{min}, k_{max}, $\sqrt{b}$) and initial litter concentrations (Table 4.3) revealed that litter quality was particularly decisive for the timing of the decomposition peak ($\sqrt{b}$). High N concentration and low C/N ratio promoted an early decomposition peak (low $\sqrt{b}$), as often demonstrated in previous studies (e.g. Edmonds, 1980; Berg et al., 1993; Aerts, 1997; Jamaludheen and Kumar, 1999; Moro and Domingo, 2000; Zhang et al., 2008). Elevated Cd or Zn concentrations in the litter, on the other hand, might have slowed down the decomposition process (Table 4.3), as can be noticed by the high $\sqrt{b}$ for aspen (Table 4.2 & Fig 4.2). A negative impact of trace metals on litter decomposition has also been found in a plethora of studies in the last decades (e.g. Tyler, 1976; Coughtrey et al., 1979; Berg et al., 1991; Laskowski et al., 1994; McEnroe and Helmisaari, 2001; Johnson and Hale, 2004). However, initial Cd and Zn concentrations in the litter did not seem to affect forest floor mass (Table 4.3). Forest floor build-up was governed by initial base cation concentrations in the litter (Table 4.3), most probably due to the positive influence of base cations on the activity of burrowing earthworms (Reich et al., 2005; Hobbie et al., 2006; De Schrijver et al., 2012).

Black locust showed the highest decomposition rate as well as the earliest start of the decomposition process (Table 4.2 & Fig 4.2). This was not surprising as black locust has shown to produce high quality litter, which is characterized by high nutrient concentrations, low C and

lignin concentrations, and consequently low C/N and lignin/N ratios (De Schrijver et al., 2012), litter properties that are generally reflected in easily decomposable litter (Berg et al., 1993; Cotrufo et al., 2000; Zhang et al., 2008; Prescott, 2010). Moreover, black locust is a N-fixing legume tree (Boring and Swank, 1984; Tateno et al., 2007; De Marco et al., 2013), known to produce N-rich litter (Jamaludheen and Kumar, 1999; Wedderburn and Carter, 1999; Moro and Domingo, 2000; Tateno et al., 2007; De Marco et al., 2013) with faster decomposition rates in the early stages compared to non-N-fixing species (Aerts and Chapin, 2000; Moro and Domingo, 2000; Tateno et al., 2007).

The fast litter mass loss for Scots pine (cfr. repeated measures ANOVA on M_t; § 4.3.1) was more surprising because coniferous litter is usually less rich in base cations and has higher C/N ratios and lignin contents (Augusto et al., 2002), litter characteristics related to slower decomposition rates (Berg et al., 1993; Cotrufo et al., 2000; Zhang et al., 2008; Prescott, 2010). Several authors demonstrated indeed that deciduous woody species decomposed significantly faster than evergreens (Cornelissen, 1996; Cornwell et al., 2008). Nevertheless, the discrepancy between fast litter mass loss from our Scots pine litterbags and its high forest floor mass (Table 4.4), was particularly striking. To some extent, confined needle litter loss through the meshes of the Scots pine litterbags was observed, and could have resulted in an overestimation of its real-world decomposition rate. Anyhow, Scots pine litter was characterized by a high initial decomposition rate as well as by an early slow-down of its decomposition rate (Fig 4.2), resulting in a considerable recalcitrant litter fraction, and thus a thick forest floor. Analogous results were found by Hobbie et al. (2006).

4.4.2 Metal amounts

Metal accumulation in or release from decomposing litter may depend on its initial metal concentrations (Lomander and Johansson, 2001; Kaila et al., 2012). We found a metal release from the 'high metal litter types' (aspen Cd and Zn; silver birch Zn) and a Cd and Zn accumulation in the 'low metal litter types' (non-accumulating *in situ* species and uncontaminated aspen and birch) (Table 4.5 & Fig 4.3).

The net accumulation suggests that the metal ions are readily fixed by the primary organic matter and/or humic substances in the leaf litter (Laskowski and Berg, 1993). Polyvalent metal ions are known for their tendency to form highly stable complexes with humic substances (Stevenson, 1982). These humic substances are produced as secondary products during the decay of organic matter. Thus, as the amount of humic substances increases in the course of a decay process, more

metal ions can be bound (Laskowski and Berg, 1993). Moreover, the stability constants for complexes formed by humic substances and some metal ions increase with increasing humification of the material (Stevenson, 1982). However, apart from immobilization of metals already present in litter by humic substances (explaining concentration increases during litter decomposition), an increase in absolute amounts obviously requires a net transport of metals into the litterbags from external sources (Laskowski et al., 1995; Lomander and Johansson, 2001). Since no measurements of the metal fluxes to and from the litterbags were conducted during our experiment, we can only speculate about sources of the influx. Accumulation of metals in unpolluted litter has been observed in previous studies (Staaf, 1980; Laskowski and Berg, 1993; Laskowski et al., 1995; Lomander and Johansson, 2001; Scheid et al., 2009; Kaila et al., 2012) and in almost all the cases this was mainly attributed to atmospheric deposition and throughfall. Another source of metal input was microbial translocation and immobilization of metals from underlying contaminated soil layers, mainly by fungi (Lomander and Johansson, 2001; Lomander, 2002; Tyler, 2005). Fungi are capable of accumulating significant amounts of metals present in their external environment (Gadd and Griffiths, 1978; Berthelsen et al., 1995), even at unpolluted sites (Lepp, 1992; Tyler, 2005). Since fungal mycelia can constitute a significant pool of organic material with a high capacity for metal accumulation, it seems likely that they can affect the translocation of metals in soil systems (Lomander, 2002). Finally, Scheid et al. (2009) determined that the main cause for metal enrichment in decomposing litter was contact of the underlying polluted soil with the litter. The Cd and Zn enrichment in our 'low metal litter types' was probably induced by a combination of above-mentioned processes.

Particularly striking was the convergence in Zn amounts between the uncontaminated and *in situ* aspen and birch litter (Fig 4.3). We believe that the Zn enrichment was mainly due to upward Zn transport from the polluted soil into the uncontaminated litter via microbiota. As Zn is an essential element, probably the microbiota are mainly contaminated with Zn, which might explain why the metal enrichment in uncontaminated aspen and birch litter was more pronounced for Zn than for Cd (see Fig 4.3 & Table 4.5). Moreover, the observed convergence in Zn amounts confirmed that metal dynamics in decomposing litter depend on the initial metal concentrations, a phenomenon that was demonstrated earlier for Zn in particular (Lomander and Johansson, 2001; Kaila et al., 2012), reflecting the Zn requirement of the decomposing heterotrophs (Gosz et al., 1973).

The fact that 'low metal litter types' became metal-enriched when they decomposed on a contaminated site, implies that the organic matter of decomposing leaves and needles acted as an efficient metal storage pool. Leaf litter can thus act as a temporary sink for metals from the soil

around and below the litter. Similar conclusions were drawn by Scheid et al. (2009); they even found that soil-derived metals that entered the litterbags were tightly bound to the decomposing leaf litter and were not mobilized during the decomposition process. Such upward metal enrichment may be ecologically relevant as litter can be transported easily to other sites (e.g. by wind) or serve as a source of food for a variety of organisms.

The decrease in metal amounts in the 'high metal litter types' (Table 4.5 & Fig 4.3) implies that Cd and Zn were released from the aspen litter and, to a lesser extent, Zn followed an analogous pattern in the birch litter. The external sources of metal input into decomposing litter, as described above, must have been negligible compared to the elevated initial metal amounts in *in situ* aspen and birch litter. Hence, metal output was faster than the input. Some studies also found metal release from decomposing litter, with higher relative releases from litter with larger initial metal amounts (Lomander and Johansson, 2001; Kaila et al., 2012), in line with our results. Such a metal release can be attributed to mineralization and might involve risks for metal dispersion from the litter towards the soil. Relations between metal releases from the 'high metal litter types' and metal amounts in the underlying topsoil and forest floor leachate are discussed below (see § 4.4.4).

4.4.3 Nutrient amounts

Our results, showing a general decrease in base cation, N and C amounts during the decomposition process (Table 4.5 & Fig 4.3), are in line with the patterns found in literature. K, Mg and Ca (here grouped as base cations) are generally known to be released during litter decomposition (e.g. Blair, 1988a; Laskowski and Berg, 1993; Laskowski et al., 1995; Brun et al., 2008; Jacob et al., 2009; Kaila et al., 2012). The high net N release from black locust (Table 4.5) was also in line with the expectations, since black locust is a N-fixing tree species (Boring and Swank, 1984) and thus characterized by litter rich in N (Table 4.1).

N dynamics in decomposing litter is generally characterized by an initial immobilization phase followed by a release, indicating that insufficient N may limit decomposer activity in early stages (e.g. Swift et al., 1979; Staaf, 1980; Berg and Staaf, 1981; Staaf and Berg, 1982; Blair, 1988a, 1988b; Prescott et al., 1993; Parton et al., 2007; Manzoni et al., 2008). Indeed, it is commonly accepted that N is usually a limiting factor for the growth of decomposer populations in forest litter (Berg and Staaf, 1987; Blair, 1988a). We observed this typical pattern of net N immobilization followed by release in all our studied litter types, except in black locust and Scots pine litter (Fig 4.3). The latter species were characterized by an immediate decline in N amounts.

Such instant N releases correspond to a leaching or rapid decay phase (Berg and Staaf, 1981), and are related to litter types that are initially rich in N (Aber and Melillo, 1982; Prescott et al., 1993; Moro and Domingo, 2000; Parton et al., 2007). Black locust indeed showed the fastest and earliest decomposition dynamics (Table 4.2 & Fig 4.2) as well as a high initial N concentration (Table 4.1), both features contributing to its instant N release. For Scots pine, however, it was unclear which processes could explain its instant N loss.

Relative releases of N after 20 months of litter decomposition were consistently less than base cation and C releases (Table 4.5), as was also found by Blair (1988a) in a mixed hardwood forest. These results indicate that the majority of base cation release occurs in the first years of decomposition, implying a rapid recycling of cation nutrients from plant litter to the soil where they may subsequently become available for plant uptake. This is in contrast to N which tends to be retained in the litter, meaning that litter may act as a sink during the first decomposition years for nutrients limiting to the decomposer community, such as N (Blair, 1988a; Manzoni et al., 2008).

4.4.4 Relations with forest floor leachate fluxes & topsoil characteristics

Metal and nutrient dynamics in decomposing litter were mainly related to topsoil characteristics, but hardly to forest floor leachate fluxes (Table 4.6 & 4.7). Only Cd leachate fluxes could be correlated to Cd dynamics in decomposing litter. Aspen was the only tree species revealing net Cd release from its litter (Table 4.5), and this resulted in higher Cd fluxes in its forest floor leachate (cfr. Table 4.6 and Fig 5.2) and elevated Cd concentrations in its topsoil (cfr. Table 4.6 & 3.6). On the other hand, net Zn release from aspen and - to a lesser extent - birch litter (Table 4.5) was not reflected in the Zn fluxes in forest floor leachates (cfr. Table 4.6 and Fig 5.2) and only partly reflected in the Zn topsoil concentrations, as Zn accumulation was only found under aspen but not under silver birch (cfr. Table 3.6). The latter might be explained by the low annual leaf litterfall amount (Table 4.4) and low Zn release (Table 4.5) under silver birch.

Net release of base cations was significantly correlated with topsoil CEC and OC content (Table 4.7). This relation was governed by black locust and aspen, both species with litter rich in base cations (Table 4.1) and characterized by high CEC and OC content in their topsoil (cfr. Table 3.5). However, nutrient releases from decomposing litter were not correlated with topsoil pH, nor with DOC and H^+ leachate fluxes. This was rather surprising as it is commonly accepted that DOC leaching and soil acidity are significantly related to litter chemistry (Reich et al., 2005; Hobbie et al., 2007; De Schrijver et al., 2012), particularly to base cation contents (Finzi et al.,

1998; Hagen-Thorn et al., 2004; Reich et al., 2005; Jacob et al., 2009). The absence of these correlations might be explained by the fact that litter decomposition measured in litterbags is never an exact representation of what happens in reality, especially when soil macrofauna is excluded (Prescott, 2005; Hobbie et al., 2006; Hättenschwiler et al., 2011), as witnesses the discrepancy between leaf litter mass loss from litterbags and forest floor mass for Scots pine.

4.5 Conclusion

In our study, litter decomposition data was fit to a model in which the decomposition rate *k* increased initially, followed by a decrease. We showed that the decomposition peak occurred within the first year for all the tree species, except for aspen. Moreover, decomposition of Scots pine, Douglas fir and silver birch litter was strongly slowed down at the end of the study period. Litter of oak, black locust and aspen, on the other hand, would have decomposed further if it was left longer in the field. During litter decomposition, the 'high metal litter types' released part of their accumulated metals, whereas the 'low metal litter types' were characterized by a net Cd and Zn enrichment. Base cations, N and C were released from all litter types as decomposition proceeded. The metal and nutrient releases from decomposing litter were mainly reflected in topsoil characteristics (Cd and Zn concentrations, CEC, OC content), but they were less clearly related to forest floor leachate fluxes (Cd, Zn, DOC, H^+). Hence, metal release from contaminated litter might involve risks for metal dispersion towards the soil. On the other hand, metal enrichment of 'low metal litter' may be ecologically relevant as it can be easily transported to other sites or serve as a food source for a variety of organisms.

5

Forest floor leachate fluxes under six tree species on a metal contaminated site

Abstract

Trees play an important role in the biogeochemical cycling of metals, although the influence of different tree species on the mobilization of metals is not yet clear. This study examined effects of six tree species on fluxes of Cd, Zn, DOC, H^+ and base cations in forest floor leachates on a metal polluted site in Belgium. Forest floor leachates were sampled with zero-tension lysimeters in a 12-year-old post-agricultural forest on a sandy soil. The tree species included were silver birch (*Betula pendula*), oak (*Quercus robur* and *Q. petraea*), black locust (*Robinia pseudoacacia*), aspen (*Populus tremula*), Scots pine (*Pinus sylvestris*) and Douglas fir (*Pseudotsuga menziesii*). We showed that total Cd fluxes in forest floor leachate under aspen were slightly higher than those in the other species' leachates, yet the relative differences between the species were considerably smaller when looking at dissolved Cd fluxes. No tree species effect was found for Zn leachate fluxes. We expected higher metal leachate fluxes under aspen as its leaf litter was significantly contaminated with Cd and Zn. We propose that the low amounts of Cd and Zn leaching under aspen's forest floor were possibly caused by high activity of soil biota, for example burrowing earthworms. Furthermore, our results revealed a significant tree species effect on H^+, DOC and base cation fluxes, with especially very low H^+ fluxes in aspen leachate (130 times less than oak). Scots pine and oak were characterized by high H^+ and DOC fluxes as well as low base cation fluxes in their forest floor leachates, implying that those species might enhance soil acidification and thus bear a potential risk for below-ground metal dispersion.

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5.1 Introduction

Metal polluted soils are often afforested in order to prevent dispersion of the metals in the environment, a technique called phytostabilization (e.g. Dickinson, 2000; Pulford and Watson, 2003; Mertens et al., 2007). Trees can potentially be very well suited for phytostabilization purposes due to their extensive root systems and high transpiration capacity (Pulford and Watson, 2003). On the other hand, tree growth might enhance metal leaching to groundwater because of soil acidification and production of dissolved organic matter (Mayer, 1998). Tree species can exert a significant influence on soil acidity and dissolved organic carbon (DOC) leaching, which has been found to be significantly related to litter quality (Reich et al., 2005; Hobbie et al., 2007; De Schrijver et al., 2012). Tree species with litter rich in calcium (Ca) were associated with lower soil acidity, increased earthworm abundance and diversity, as well as higher forest floor turnover rates (Reich et al., 2005; Hobbie et al., 2006; Jacob et al., 2009; De Schrijver et al., 2012). In contrast, species producing litter with low contents of base cations and high concentrations of organic acids have been shown to decompose slowly and enhance soil acidification and DOC leaching (Konova, 1966; Finzi et al., 1998; Hagen-Thorn et al., 2004).

Tree species producing contrasting leaf litter in terms of chemical composition and degradability thus have a different influence on the composition and reactivity of the forest floor leachate and the soil solution (Strobel et al., 2001a). Forest floor leachate composition is highly relevant, in particular the fluxes of DOC and protons (H^+) as these are expected to increase metal leaching. Numerous studies have shown that the forest floor is the major source of DOC in forest ecosystems (Cronan and Aiken, 1985; Qualls et al., 1991; Currie et al., 1996; Michalzik and Matzner, 1999; Michalzik et al., 2001; Vandenbruwane, 2008). DOC is one of the most actively cycling soil organic carbon (C) pools and its significance in forest ecosystems has been highlighted because of its ecological function in transport and cycling of nutrients and metals in soils (Qualls and Haines, 1991; Qualls et al., 1991; Tipping and Hurley, 1992; Michalzik and Matzner, 1999; Kalbitz et al., 2000). Tree species producing forest floor leachate with high DOC concentrations are stimulating base cation (Finzi et al., 1998) and metal leaching (Strobel et al., 2001a). Leaching of base cations causes soil acidification (Marschner, 1995; Finzi et al., 1998), concomitantly increasing metal mobilization in the soil (e.g. McBride et al., 1997; Sauvé et al., 2000a, 2000b; Voegelin et al., 2003). Moreover, the presence of base cations in the soil solution may also increase the mobility of some metals: it was shown that increased Ca^{2+} concentrations in the soil solution significantly displace Cd^{2+} from sorption sites (Christensen, 1984; Temminghoff et al., 1995). Metal leaching to groundwater poses serious risks for ecosystems and human health (WHO, 2000).

The potential feedback mechanisms between metal accumulation in trees, leaf litter quality, the composition of the forest floor leachate and the soil solution thus all affect metal mobility. Therefore, metal mobility is likely to be strongly species dependent and strategies for phytostabilization of metals need to carefully consider the importance of tree species selection. Here we aim to unravel the tree species effects on fluxes of Cd, Zn, DOC, H^+ and base cations in forest floor leachate, with respect to Cd and Zn mobilization. For estimating the risk of Cd and Zn leaching to deeper soil layers, the H^+, base cation and metal fluxes in the forest floor leachates are of particular interest. Nevertheless, to our knowledge, no field studies incorporating fluxes and interrelationships of DOC, H^+, base cations and metals in forest floor leachates exist. Furthermore, we found no field studies comparing metal leaching from the forest floor under different tree species.

We hypothesize that (i) tree species with low litter quality (in terms of base cations and C/N ratio) produce high DOC and H^+ and low base cation fluxes in their forest floor leachates, enhancing soil acidification and thus eventually causing risk for metal leaching and (ii) tree species with high metal concentrations in their leaf litter give rise to higher metal fluxes in their forest floor leachates, also causing risk for below-ground metal dispersion.

5.2 Materials and Methods

5.2.1 Experimental set-up and sampling

The study included the six considered tree species (oak, silver birch, black locust, aspen, Scots pine, Douglas fir) and was executed in the selected stands in 'Waaltjesbos' (see Fig 3.1). See § 3.2.1 for full site descriptions and stand selection. A characterization of the soil of the study site is given in Table 3.5 (soil characteristics pH-KCl, OC content, CEC) and Table 3.6 (total and extractable soil Cd and Zn concentrations). The six tree species have divergent leaf litter quality and show different metal concentrations in their leaf litter (see Tables 4.1 and 5.1). Hence, forest floor leachates under these tree species are expected to reflect the biogeochemical interactions that took place over the preceding years.

In each selected stand, two zero-tension lysimeters were installed to collect forest floor leachate. The zero-tension lysimeters were constructed from 30 cm long PVC guttering (324 cm^2) covered with wire netting (1.5 mm mesh size). Each lysimeter was connected with flexible PVC tubing to a 2-l polyethylene (PE) bottle installed beneath the lysimeter. The bottles were placed below ground level to avoid the growth of algae and to keep the samples cool. The lysimeters were

installed directly under the forest floor (Ol + Of if present). The Of horizon had started to develop in most of the stands, except in the black locust and Douglas fir stands, but an Oh horizon was not present yet under this young forest (Jabiol et al., 1995). To ensure optimal drainage from the gutter into the bottle, lysimeters were placed under a slight inclination.

Installation of a zero-tension lysimeter (upper) and sampling of the forest floor leachate (lower)

Leachates were sampled three-weekly between September 2009 and September 2010. The water volumes were measured in the field, and 300-ml subsamples of the forest floor leachates were taken to the lab for chemical analysis. The samples were transported in a cooler, once in the lab they were stored in a freezer until analysis. At every sampling event, the PE sampling bottles were replaced by specimens that were rinsed with distilled water.

5.2.2 Sample analysis

Forest floor leachates from different sampling occasions were pooled volume-weighted to bulk samples per season (autumn: 19/09/2009 – 30/11/2009; winter: 01/12/2009 – 31/03/2010; spring: 01/04/2010 – 24/06/2010; summer: 25/06/2010 – 21/09/2010). Prior to analyses, bulk samples were filtered through a 0.45 µm nylon filter (Gelman), except for pH and total Cd and Zn concentrations, which were measured on the unfiltered samples. pH was measured with a pH

Orion electrode; hydrogen ion (H^+) concentration was calculated from the measured pH value. Total Cd and Zn concentrations were measured with ICP-MS (Perkin-Elmer Elan DRC-e) and ICP-OES (Varian Vista-MPX) respectively, after a destruction with HNO_3 and H_2O_2. Filtered samples were analyzed for the base cations Ca, potassium (K), magnesium (Mg) and sodium (Na) (flame atomic absorption spectrophotometry, Varian SpectrAA-240), for DOC (Total Organic Carbon Analyzer, Shimadzu TOC-VCPN) and for dissolved Cd and Zn (respectively ICP-MS, Perkin-Elmer Elan DRC-e and ICP-OES, Varian Vista-MPX).

In order to get insight in the leaf litterfall amounts and chemical composition, leaf litter samples were collected with litterfall traps in 2007-2008 (see § 3.2.2 and § 3.2.3), weighed and dried at 70 °C to constant weight and ground using a centrifugal mill (Retsch ZM1, Germany). Subsequently, ground samples were analyzed for Cd, Zn, Ca, K, Mg, Na, C and nitrogen (N). The samples were ashed at 450 °C and dissolved in HNO_3 before Cd and Zn analysis using ICP-MS (Elan DRC) and ICP-OES (Varian Vista-MPX) respectively. The K, Ca, Mg, Na concentrations were measured by means of flame atomic absorption spectrophotometry (Varian SpectrAA-240) after a destruction in HNO_3-$HClO_4$. C and N concentrations were determined by elemental analysis (Vario Max CNS, Germany).

5.2.3 Water fluxes

Water fluxes in the forest floor were not calculated from volumes collected in the zero-tension lysimeters as these volumes were highly variable from collector to collector, ranging from 1.3% to 87% of open-field precipitation (P). Several authors recognized the great variability of water volumes collected with lysimeters, and suggested to estimate the water fluxes indirectly (Haines et al., 1982; Fröberg et al., 2005; Kalbitz et al., 2007; Landre et al., 2010; Nieminen, 2011). Consequently, water fluxes in the forest floor were considered to be equal to throughfall (TF) fluxes (Fröberg et al., 2005). However, the forest floor will intercept and evaporate a fraction of throughfall (Putuhena and Cordery, 1996). A few studies describe forest floor evaporation in relation to throughfall flux. Some found a tight resemblance between throughfall flux and water flux in the forest floor, with only 1% (Vandenbruwane, 2008) or 2% (Qualls et al., 1991) of the throughfall being absorbed by the forest floor and later evaporated, supporting our assumption. Others, on the other hand, saw that evaporation from the forest floor contributed an important share to the forest water balance, ranging from 20% in the Ol-horizon of a Norway spruce forest (Kalbitz et al., 2004) over 22% in a beech forest (Solinger et al., 2001; Gerrits et al., 2010), up to 26% in a mixed deciduous forest (Bittner et al., 2010) of the throughfall flux. However, as our study site is characterized by a thin forest floor (see § 5.2.1), we assume that the applied method

(cfr. Fröberg et al., 2005) is adequate, bearing in mind that the throughfall fluxes will slightly overestimate the actual water fluxes below the forest floor.

In order to measure throughfall fluxes, four throughfall collectors were installed randomly in every stand, consisting of PE funnels (154 cm^2) draining into 2-l PE bottles below ground level. Bulk precipitation was measured in an open patch in the forest by means of six bulk collectors, similar to the throughfall collectors. Stemflow was not collected because of its negligible contribution to the total water flux reaching the forest floor in this young forest (see also Alcock and Morton, 1985; Grelle et al., 1997; Price et al., 1997; Bergholm et al., 2003; Bittner et al., 2010). Water volumes in throughfall and bulk collectors were measured three-weekly from April 2011 until April 2012. Species-specific canopy interception loss (I_c) was calculated as (P – TF)/P, according to the growing (May – October) and the dormant season (November – April). Precipitation data of September 2009 - September 2010 (data of the Royal Meteorological Institute of Belgium) were multiplied with 1 – I_c to calculate species-specific throughfall fluxes during the period when forest floor leachates were sampled.

Throughfall collectors (left) and bulk collectors (right)

5.2.4 Data analysis

Leachate fluxes were calculated by multiplying water fluxes by leachate concentrations. The sum of these seasonal leachate fluxes over the one year monitoring period yielded annual leachate fluxes. Base cation fluxes were calculated by summing the equivalents of K^+, Ca^{2+}, Mg^{2+} and Na^+. Base cation fluxes, together with leachate pH and fluxes of DOC, total and dissolved Cd and Zn and H^+ were considered as response variables.

First, both the intercorrelations between the seasonal leachate fluxes of Cd, Zn, base cations, DOC, H^+ and pH, and the correlations between those seasonal leachate fluxes and leaf litter characteristics (Cd and Zn amounts, base cation amounts (sum of K^+, Ca^{2+}, Mg^{2+}, Na^+ equivalents) and C/N ratio) were calculated by means of Spearman correlation coefficients.

The experimental design was hierarchical, with lysimeters grouped within stands and tree species replicated at the stand level, so we used multilevel models. The lmer function in the lme4 library of R version 2.13.2 was used here (Bates et al., 2011; R Core Team, 2014). First, we examined whether there was a species and/or a seasonal effect on the leachate fluxes. Therefore, we constructed a null model with two random effect terms: the 'Stand' that was sampled and the 'Lysimeter code' nested within stand, which accounted for the repeated measurements on the same lysimeter. Next, we tested models with the explanatory factor 'Species' or 'Season' as fixed effects against the null model with a likelihood ratio test to evaluate whether these factors significantly improved the null model. The factor 'Season' was included in the fixed effects, but we allowed the seasonal effect to vary by lysimeter and allowed for correlation among the seasonal effects for the same lysimeter. Finally, a full factorial model with 'Species' and 'Season' as fixed effects was fitted using restricted maximum likelihood estimates. Prior to all multilevel analyses, log transformations were applied to maximize the normality of the data.

5.3 Results

5.3.1 Leaf litter characteristics

Leaf litter characteristics have a direct link with the forest floor leachate. Aspen clearly took up more Cd than the other species, with the Cd concentration in the leaf litter being about 7 to 12 times higher than that of the other tree species. Zn concentrations in leaf litter were significantly higher in aspen and silver birch. Highest base cation concentrations were found for aspen and black locust, while Scots pine showed the highest C/N ratio (Table 5.1).

Leaf litter concentrations (Table 5.1) were multiplied by annual leaf litterfall to calculate the amounts (fluxes) of metals and base cations reaching the forest floor with litterfall (Table 5.2). N was not further considered as it was not taken into account for the leachate fluxes. C was not further considered as C amounts were not taken into account for the correlations between leachate fluxes and leaf litter characteristics (only C/N ratio).

Cd and Zn litterfall fluxes were highest for aspen, due to its high Cd and Zn concentrations (Table 5.1) and high leaf litter production (Table 5.2). Silver birch, on the other hand, showed high Zn concentrations in its leaf litter (Table 5.1), but the Zn amount did not differ from the other species due to its very low leaf litter production (Table 5.2). A similar pattern was found for the base cations: highest fluxes were found under aspen, as this species showed high base cation concentrations (Table 5.1) as well as high leaf litter production, whereas black locust, also showing high base cation concentrations, did not exhibit a high base cation amount due to its low leaf litter production (Table 5.2).

Table 5.1 Cd, Zn, base cations, C and N concentrations and C/N ratio in leaf litter of the different tree species (average ± st.dev); differences between species were tested using analysis of variance (ANOVA) with a Bonferroni post hoc test, values with the same letter did not differ between species ($p < 0.05$)

	Cd [§] (mg kg^{-1})	Zn [§] (mg kg^{-1})	BC [§] (meq kg^{-1})	C [§] (%)	N [§] (%)	C/N (-)
silver birch	1.72 ± 0.44 [a]	1 540 ± 341 [b]	779 ± 124 [a]	46.8 ± 0.3 [bc]	1.50 ± 0.07 [a]	31.3 ± 1.4 [a]
oak	< 1.0 [a]	287 ± 106 [a]	798 ± 86 [a]	46.5 ± 0.4 [b]	1.56 ± 0.19 [a]	30.1 ± 3.8 [a]
black locust	1.22 ± 0.40 (1) [a]	427 ± 100 [a]	1477 ± 188 [b]	44.7 ± 0.2 [a]	2.29 ± 0.30 [b]	19.8 ± 2.9 [a]
aspen	13.0 ± 6.5 [b]	2 354 ± 494 [c]	1585 ± 291 [b]	45.1 ± 0.3 [a]	1.48 ± 0.32 [a]	31.6 ± 7.3 [a]
Scots pine	1.08 ± 0.34 (1) [a]	321 ± 56 [a]	619 ± 56 [a]	47.7 ± 0.3 [c]	1.04 ± 0.01 [a]	45.9 ± 0.6 [b]
Douglas fir	< 1.0 [a]	295 ± 22 [a]	639 ± 148 [a]	47.5 ± 0.5 [bc]	1.62 ± 0.40 [ab]	30.2 ± 7.1 [a]

[§] Concentrations analyzed on leaf litter samples (n = 6) collected with litterfall traps in 2007-2008 (cfr. § 3.2.2)

< if all values were lower than the determination limit, the determination limit was given, preceded by '<'

(1) the number between brackets is the number of values lower than the determination limit of 1.0 for Cd. The mean was calculated by equalling those values to the half of the determination limit.

Table 5.2 Annual leaf litterfall M_{LF} and amounts (fluxes) of Cd, Zn and base cations in leaf litterfall of the six tree species (average ± st.dev); differences between species were tested using analysis of variance (ANOVA) with a Bonferroni post hoc test, values with the same letter did not differ between species ($p < 0.05$)

	M_{LF} [§] (ton ha^{-1} yr^{-1})	Cd (g ha^{-1} yr^{-1})	Zn (kg ha^{-1} yr^{-1})	BC (eq ha^{-1} yr^{-1})
silver birch	1.4 ± 0.6 [a]	2.4 ± 1.3 [a]	2.2 ± 1.1 [a]	1092 ± 527 [a]
oak	3.7 ± 0.7 [cd]	1.9 ± 0.3 [a]	1.1 ± 0.4 [a]	2956 ± 635 [b]
black locust	2.1 ± 1.0 [ab]	2.5 ± 1.5 [a]	0.9 ± 0.5 [a]	3087 ± 1590 [b]
aspen	4.0 ± 1.0 [cd]	52.4 ± 29.4 [b]	9.5 ± 3.1 [b]	6370 ± 2019 [c]
Scots pine	4.9 ± 1.1 [d]	5.3 ± 2.0 [a]	1.6 ± 0.5 [a]	3010 ± 746 [b]
Douglas fir	3.1 ± 1.3 [bc]	1.5 ± 0.7 [a]	0.9 ± 0.4 [a]	1969 ± 972 [ab]

[§] M_{LF} estimated by weighing the leaf litter collected in the litterfall traps (n = 12) in 2007-2008 (cfr. Table 3.2)

5.3.2 Forest floor leachate

Leachate pH and seasonal concentrations of Cd, Zn, DOC and base cations in forest floor leachates are reported in Appendix A.1. However, here we focus on the leachate fluxes (amounts) of Cd, Zn, DOC and base cations, since fluxes are more important for the discussion of biogeochemical cycles. Leachate fluxes were estimated by multiplying leachate concentrations by water fluxes in the forest floor. Seasonal and annual leachate fluxes are reported in Appendix A.2.

Water fluxes

Water fluxes in the forest floor showed a strong seasonal pattern: spring 2010 was extremely dry, whereas the summer of 2010 was very wet (Fig 5.1). Moreover, the water fluxes varied under the different tree species, which was attributed to a significant species effect on interception values. Canopy interception loss (I_c) was significantly higher for the coniferous species compared to the broadleaved species. I_c values ranged from 10.1 % (black locust) over 10.6 % (oak), 18.3 % (silver birch), 26.4 % (aspen), 43.6 % (Douglas fir) up to 56.5 % (Scots pine).

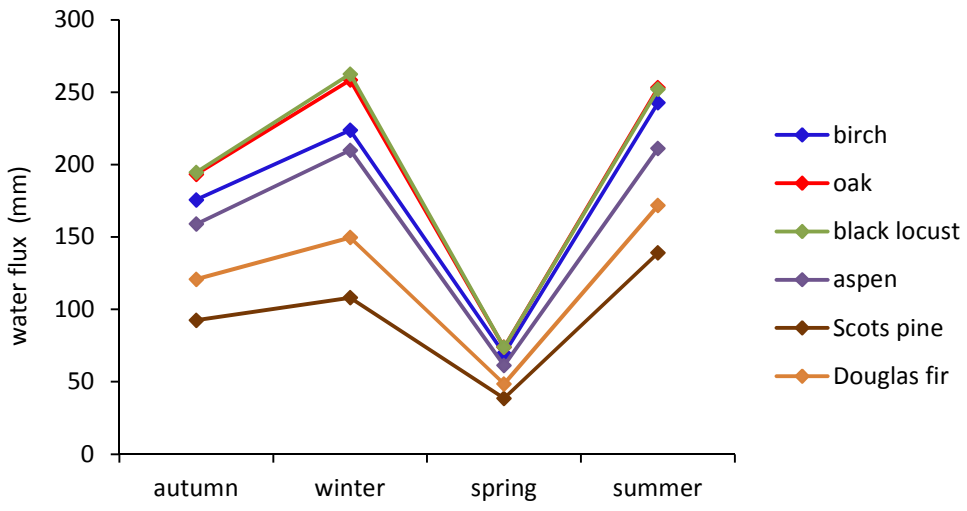


Figure 5.1 Estimated water fluxes in the forest floor under the six tree species

Leachate fluxes

As one could expect, the seasonal effect on forest floor leachate fluxes was pronounced for all response variables (Table 5.3), according to the seasonally changing water fluxes. Moreover, there was a significant species effect on forest floor leachate fluxes for all response variables, except for Zn (Table 5.3). This species effect was examined more in detail by comparing annual leachate fluxes (Fig 5.2, see also Appendix A.2).

Table 5.3 Contribution of 'Species' and 'Season' on leachate fluxes of total and dissolved Cd and Zn, DOC, H^+ and base cations. The likelihood ratio χ^2 test statistics represent comparisons of a multilevel model without fixed effects and a model with 'Species' or 'Season'. See methods for details on the model building.

	Cd_{tot} §	Cd_{diss} §	Zn_{tot} §	Zn_{diss} §	DOC §	H^+ §	BC §
Likelihood ratio χ^2							
Species	19.6 **	17.3 **	6.5	8.1	22.0 ***	27.2 ***	31.2 ***
Season	54.2 ***	89.0 ***	90.0 ***	87.2 ***	98.7 ***	35.5 ***	88.7 ***

Full factorial model R syntax: Response ~ -1 + Species + Season + (1 | Stand) + (Season - 1 | Stand:Code)

§ log transformed; ** $p < 0.01$; *** $p < 0.001$

Total Cd fluxes in forest floor leachate under aspen were higher than those under oak, Scots pine, Douglas fir and silver birch, the latter two species showing the lowest Cd fluxes (Fig 5.2). The dissolved Cd fluxes in aspen leachate were only distinguished from those under silver birch (Fig 5.2). No tree species effect was found for Zn fluxes in forest floor leachate (Fig 5.2), in spite of significant differences in Zn amounts in leaf litter (Table 5.2) and a positive correlation between Zn leachate fluxes and Zn litter amounts (Table 5.4).

Highest base cation fluxes in forest floor leachate were found under aspen and black locust (Fig 5.2), significantly correlated with base cation litter amounts and negatively correlated with the C/N ratio of the leaf litter (Table 5.4). Ca^{2+} and K^{+} were the dominant cations in the leachate for all considered tree species (Fig 5.2).

Scots pine and oak induced significantly higher DOC fluxes compared to silver birch, aspen and Douglas fir (respectively 1.7, 1.5 and 1.4 times higher), while black locust produced intermediate DOC fluxes (Fig 5.2). A similar pattern was found for H^{+} fluxes, with especially very low values in aspen leachate (130 times less than oak) (Fig 5.2). DOC and H^{+} leachate fluxes were significantly intercorrelated (Table 5.5). Surprisingly, neither DOC nor H^{+} leachate fluxes were correlated with base cation amounts or with the C/N ratio of the leaf litter (Table 5.4).

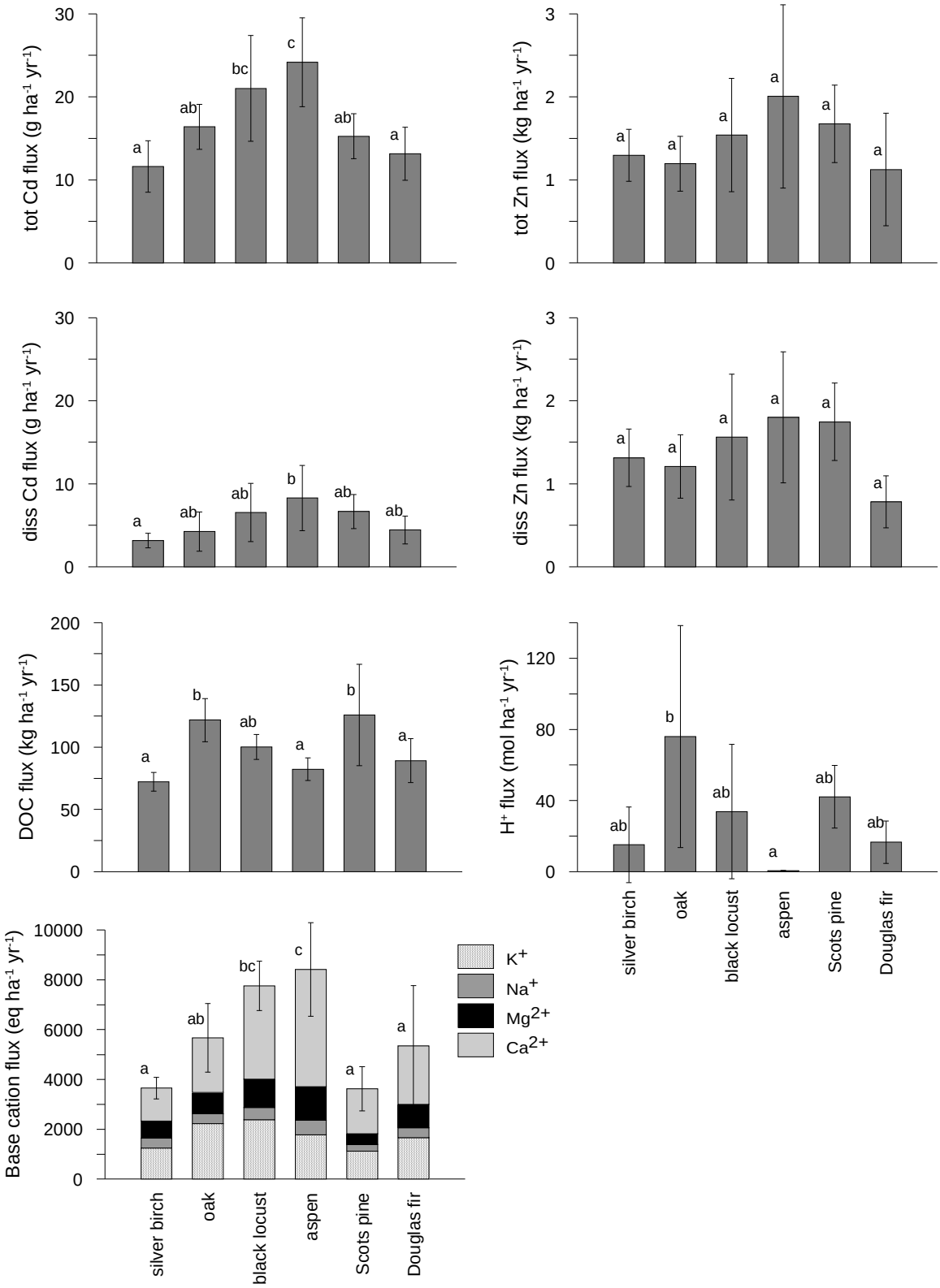


Figure 5.2 Annual fluxes of total Cd and Zn, dissolved Cd and Zn, DOC, H^+ and base cations in forest floor leachate under the six tree species (average ± st.dev); differences between species were tested using a multilevel model with a Bonferroni post hoc test, values with the same letter did not differ between species ($p < 0.05$)

Table 5.4 Spearman's ϱ correlation coefficients between annual leachate fluxes (rows) and leaf litter characteristics (Cd, Zn and base cation amounts and C/N ratio; columns) (n = 36)

	Cd	Zn	BC	C/N
Cd_{tot}	0.480 **	0.141	0.658 **	- 0.197
Cd_{diss}	0.401 *	0.139	0.460 **	0.045
Zn_{tot}	0.459 **	0.380 *	0.262	0.131
Zn_{diss}	0.588 **	0.405 *	0.306	0.222
DOC	0.032	- 0.377 *	0.253	- 0.011
H^+	- 0.352 *	- 0.558 **	- 0.194	0.107
BC	0.226	- 0.027	0.497 **	- 0.516 **

* $p < 0.05$; ** $p < 0.01$

Table 5.5 Spearman's ϱ correlation coefficients of the intercorrelation between annual leachate fluxes (n = 36)

	Cd_{tot}	Cd_{diss}	Zn_{tot}	Zn_{diss}	DOC	H^+
Cd_{tot}						
Cd_{diss}	0.638 **					
Zn_{tot}	0.582 **	0.671 **				
Zn_{diss}	0.531 **	0.694 **	0.837 **			
DOC	0.264	0.179	0.232	0.174		
H^+	- 0.126	- 0.088	- 0.071	- 0.047	0.426 **	
BC	0.601 **	0.403 *	0.269	0.161	0.259	- 0.287

* $p < 0.05$; ** $p < 0.01$

5.4 Discussion

In order to get insight in the risk tree species exert on metal mobilization in metal polluted soils, we examined forest floor leachates under different tree species with diverging leaf litter quality. Litter decomposition is mainly affected by climate, soil and vegetation (Meentemeyer, 1978). Here, the selected stands developed in homogenous conditions of climate and soil, as they are all part of a relatively small forest (203 ha, see also § 3.2.1). Differences in litter decomposition products (i.e. forest floor leachates) are thus closely linked with tree species.

Leachate fluxes were calculated by means of the estimated water fluxes in the forest floor, which were considered to be equal to throughfall fluxes. As these estimated water fluxes probably are a slight overestimation of the actual water fluxes (see § 5.2.3), one should bear in mind that absolute values of the forest floor leachate fluxes should be interpreted with care. However, the relative differences between tree species are most relevant in the scope of this study, and we consider these to be reliable.

5.4.1 Fluxes of H^+, DOC and base cations

Metal solubility, and hence mobility, is generally enhanced by acidity and by complexation with dissolved organic matter (McBride et al., 1997; Sauvé et al., 2000a, 2000b; Weng et al., 2002; Strobel et al., 2005). Therefore, H^+ and DOC fluxes in forest floor leachates are of major concern as they reflect the amounts of protons and DOC entering the mineral soil, where they might mobilize accumulated metals. Many authors found that DOC particularly has a strong affinity for Cu, Ni, Hg and Pb, whereas Cd and Zn tend not to complex strongly with soluble organics. This implies that the solubility of Cd and Zn is mainly controlled by pH with minor to no effects of DOC (e.g. Bergkvist et al., 1989; Holm et al., 1995; Römkens and de Vries, 1995; McBride et al., 1997; Sauvé et al., 2000b; Weng et al., 2001b; Strobel et al., 2001b, 2005).

Our results showed a significant tree species effect on H^+ fluxes, with especially very low values in aspen leachate (Fig 5.2). Given the univocal effect of pH on metal solubility (e.g. Sauvé et al., 2000a, 2000b; Degryse et al., 2007), we expect that the extremely low proton amounts leaching from aspen's forest floor (130 times less than oak) will result in low Cd and Zn mobilization in the (top)soil under aspen trees. This was confirmed by the immediately bioavailable ($CaCl_2$-extractable) Cd and Zn concentrations, that appeared not to be elevated in the topsoil under aspen, despite significant accumulation of total Cd and Zn (see Table 3.6).

In analogy to H^+ fluxes, a similar pattern was found for DOC fluxes. The positive intercorrelation between DOC and H^+ fluxes in forest floor leachates (Table 5.5) was expected as soil acidification is known to be enhanced by species with high litter concentrations of organic acids (Finzi et al., 1998). Scots pine and oak induced significantly higher DOC fluxes compared to silver birch, aspen and Douglas fir (Fig 5.2). Several studies revealed higher DOC fluxes in forest floor leachate from coniferous species compared to broadleaved species (Cronan and Aiken, 1985; Currie et al., 1996; Fröberg et al., 2011; Lindroos et al., 2011). Coniferous litter often is less rich in base cations and has higher C/N ratios and lignin contents (Augusto et al., 2002), litter characteristics related to slower decomposition rates (Cotrufo et al., 2000; Hobbie et al., 2006; Prescott, 2010). Consequently, coniferous litter is often more resistant to biological degradation and leaches more organic acids compared to broadleaved litter (Howard and Howard, 1990; Ranger and Nys, 1994; Johansson, 1995). However, Michalzik et al. (2001) concluded from a synthesis of 42 field studies from temperate forests that annual fluxes of DOC in deciduous forests were within the range of those from coniferous sites, at least on a regional scale. Also Vandenbruwane (2008) did not find a difference in DOC fluxes under the forest floor of a Corsican pine and a silver birch stand. In a common garden study, DOC release from the litter layer (Ol-horizon) was significantly higher under oak than under Douglas fir (Trum et al., 2011), in line with our results.

In temperate forest ecosystems, fluxes of DOC from the forest floor into the mineral soil have been estimated at 100-400 kg C ha^{-1} yr^{-1} (Michalzik et al., 2001). Our results are on the lower side (65-170 kg ha^{-1} yr^{-1}), most likely because it is a young post-agricultural forest (ca. 12 years) and a well-developed forest floor is not present yet (absence of Of- and Oh-horizon, see § 5.2.1). The former agricultural practices of fertilization and liming, resulting in a higher nutrient status and soil pH, are favorable for both microbial and earthworm communities (Edwards, 2004; Fierer and Jackson, 2006), being beneficial for litter decomposition rates (Reich et al., 2005), which in turn results in lower DOC production (Konova, 1966; Finzi et al., 1998). For species with slowly decomposing litter, increasing forest age will cause a build-up of the forest floor (De Schrijver et al., 2012). Therefore, we here suggest that DOC and H^+ leaching from the forest floor will further increase under the tree species with slower decomposing litter, as oak and pine, whereas it will stay more or less unaltered under aspen as a consequence of its fast litter decomposition (see also further). Under black locust we expect DOC and H^+ leaching to increase with forest age, given black locust's ability to symbiotically fix atmospheric N_2. This process can enhance soil acidification as a by-product of increased nitrification rates (Van Miegroet and Cole, 1984), as was found for *Alnus glutinosa* and *Alnus rubra* in other studies (Van Miegroet and Cole, 1984,

1985; Compton et al., 2003; De Schrijver et al., 2012). After 35 years of post-agricultural forest development, *A. glutinosa* developed a thick forest floor and this despite high base cation contents in its leaf litter (De Schrijver et al., 2012), probably causing high DOC leaching. We might expect that the latter will also occur in the black locust stands at our study site. Further observations are needed to confirm this.

Base cations entering the soil via forest floor leachate may have a dual effect on metal mobilization in the (top)soil. On the one hand they can be expected to play a significant role in preventing metal leaching as they are of relevance for buffering protons and preventing soil acidification (Marschner, 1995; Finzi et al., 1998). On the other hand, base cations in the soil solution may also increase the mobility of some metals: it was shown that increased Ca^{2+} concentrations in the soil solution significantly displace Cd^{2+} from sorption sites (Christensen, 1984; Temminghoff et al., 1995). It was not clear which of both processes was decisive at our site.

Base cations release from the forest floor is species-specific as it is favored by leaf litter with higher degradability and better quality (fast litter decomposition rate) (Hagen-Thorn et al., 2004; Jacob et al., 2009). Highest base cation fluxes in forest floor leachate were found under aspen and black locust (Fig 5.2), being correlated to base cation amounts in the leaf litter (Table 5.4), even though the base cation amount in black locust's leaf litterfall was not elevated compared to the other species (Table 5.2). Due to its low leaf litter production (Table 5.2), the high base cation concentration in black locust's leaf litter (Table 5.1) was not reflected in a high base cation amount in its leaf litterfall (Table 5.2). However, since black locust and aspen litter have shown to be easily decomposable (Aerts and Chapin, 2000; Cooke and Weih, 2005), both species promote a fast base cation return to the soil, explaining the highest base cation fluxes in humus leachates under these species.

Summarized, with respect to Cd and Zn mobilization, aspen achieved the best results as it generated extremely low H^+ fluxes in its forest floor leachate. It seems that aspen's high base cation leachate fluxes also contributed to stabilization of Cd and Zn: despite significant accumulation of total Cd and Zn concentrations in its topsoil, we found that its immediately bioavailable ($CaCl_2$-extractable) Cd and Zn topsoil concentrations were not elevated (see Table 3.6). This made us assume that the metal-stabilizing effect of the base cations entering the soil via forest floor leachates (by buffering protons and hence counteracting soil acidification) was relatively more decisive than their mobilizing effect (by competing with metal ions for sorption sites). Scots pine and oak, on the contrary, were the inferior species with respect to Cd and Zn

mobilization, because of high H^+ fluxes as well as low base cation fluxes in their forest floor leachates. Douglas fir, black locust and silver birch scored intermediately.

5.4.2 Fluxes of Cd and Zn

Although the Cd flux through leaf litterfall in aspen stands was 10 to 34 times higher than the litterfall fluxes of Cd under the other studied species (Table 5.2), the total Cd fluxes via forest floor leachate were only 1.2 to 2.1 times higher under aspen (see Fig 5.2). In analogy to Cd fluxes, we had expected higher Zn leachate fluxes under aspen since the Zn flux through leaf litterfall in aspen stands was 4.4 to 10.6 times higher compared to the other tree species (Table 5.2).

We here propose that the low amounts of Cd and Zn leaching under aspen's forest floor are possibly caused by soil biotic activity, showing divergent behavior under different tree species. Burrowing earthworms, for example, were shown to pull significant amounts of leaf litter in the soil (Edwards, 2004; Reich et al., 2005). Although we did not sample the earthworm populations in this forest, recent studies (Reich et al., 2005; Jacob et al., 2009; De Schrijver et al., 2012) revealed that tree species with high leaf litter Ca concentrations are beneficial for the amounts and species composition of earthworms, causing permanent mixing of topsoil with litter, earthworm excrements and soil from deeper soil layers. As such, burrowing earthworms counteract soil acidification (De Schrijver et al., 2012). Earthworm sampling in a 38-yr-old post-agricultural forest showed a significantly higher biomass and richness of burrowing earthworm species under a poplar species (*Populus x euramericana*) compared to six other broadleaved tree species (unpublished results). The significantly higher contents of organic matter (cfr. OC content), the higher CEC and soil pH found in the topsoil under aspen at our study site (see Table 3.5) are confirming our hypothesis of high earthworm amounts under aspen (in line with Reich et al., 2005).

Relative differences between the tree species were even smaller when looking at dissolved Cd fluxes (Fig 5.2). Leachate pH under aspen was significantly higher compared to the other species (see Appendix A.1), resulting in low dissolved Cd fluxes in aspen leachate. This finding is consistent with the results of Huang et al. (2011), who found that Cd was mobilized from the forest floor dominantly during episodes of lower pH, in a Norway spruce (*Picea abies*) forest. However, as total Cd fluxes in aspen leachate were elevated compared to the other species, it indicates that there is a (soluble) fraction of Cd in aspen's leachate which might become dissolved, and thus prone to leaching to deeper soil layers or groundwater, in more acid conditions.

The elevated input of Cd and Zn via leaf litterfall is giving rise to higher total Cd and Zn concentrations in the topsoil under aspen compared to the other tree species and to deeper soil layers (see Table 3.6). Aspen trees thus circulate significant amounts of Cd and Zn via their leaf litter, yet this is not univocally reflected in higher metal fluxes in their forest floor leachates, especially not when considering dissolved metal fluxes. These findings were thus in contrast with our hypothesis (ii), namely that tree species with high metal concentrations in their leaf litter give rise to higher metal fluxes in their forest floor leachates. The observed Cd and Zn accumulation in the topsoil under aspen might be caused by high activity of burrowing earthworms, mixing the topsoil with contaminated litter. Our results, showing that metal leaching from the forest floor under aspen was not as high as foreseen, might imply that the risks of below-ground metal dispersion under aspen might actually be smaller than expected. More research on this issue is done in Chapter 7.

As only input of elements via litterfall was measured, the total element input to the forest floor was underestimated. The sum of element fluxes via throughfall (TF) and litterfall (LF) constitutes the total element input to the forest floor and includes both external (atmospheric) and internal (uptake from soil and recycling through litterfall and foliar leaching) sources (Ukonmaanaho et al., 2001). Metal emissions from the factory nearby have been almost eliminated during the past decades and therefore we did not measure metal fluxes via TF. However, secondary deposition of Cd and Zn, due to resuspension of contaminated soil, may nowadays contribute to metal input to the forest floor and (top)soil (Seuntjens, 2002; van der Grift and Griffioen, 2008). Average Cd concentrations in the air, measured from 2005 until 2008 in the close vicinity of our study site (data of the Flemish Environment Agency), were far below the European target value for Cd (5 ng m^{-3}). However, tentative estimations, based on average Cd and Zn concentrations in the air and deposition velocity, indicated that TF fluxes of Cd and Zn were considerable. Aspen was the only species where LF fluxes of Cd and Zn largely exceeded the estimated TF fluxes. For the other tree species, the Cd flux through TF was estimated to be higher than through LF, whereas for Zn both input fluxes showed an equal share. Moreover, our estimations suggested that Cd TF fluxes might account for the greater part of the discrepancy between Cd fluxes in litterfall (Table 5.2) and those in forest floor leachates (Fig 5.2) for the tree species that did not accumulate Cd in their leaves. This insight emphasizes the relevance of TF measurements within future research.

5.5 Conclusions and outlook

Tree species can exert a significant influence on metal mobility, through leaching of H^+, DOC, base cations and/or metals from the forest floor. After all, forest floor leachate chemistry determines to a large extent the biogeochemical processes in the soil and the soil solution chemistry, thus affecting metal mobilization and below-ground dispersion risks.

Our results revealed aspen, silver birch and Douglas fir as species with the lowest H^+ and DOC fluxes (Fig 5.2), at least at this young stage of forest development. With regard to the base cation fluxes, aspen and black locust showed the highest fluxes (Fig 5.2). Hence, considering H^+, DOC as well as base cation fluxes, it seems that aspen can minimize metal mobility in the (top)soil, whereas oak and Scots pine might enhance (top)soil acidification, and thus bear a potential risk for below-ground metal dispersion. Douglas fir, black locust and silver birch seem to be intermediate options, although we expect the H^+ and DOC fluxes in their forest floor leachates to increase significantly with increasing forest development (see also § 5.4.1).

The preceding results were confirmed by the immediately bioavailable ($CaCl_2$-extractable) Cd and Zn concentrations, that appeared not to be elevated in the topsoil under aspen, despite significant accumulation of total Cd and Zn (see Table 3.6). For Scots pine and oak, however, no significant changes in total or immediately bioavailable Cd and Zn topsoil concentrations, compared to deeper soil layers, were found (yet) in this young forest.

Leaf litter of aspen is contaminated with Cd and Zn (Tables 5.1 and 5.2), resulting in a significant Cd and Zn accumulation in the topsoil under aspen (see Table 3.6). For that reason we previously recommended to avoid *Populus* species when afforesting Cd and Zn contaminated lands (Mertens et al., 2007; see also Chapter 3). Moreover, total Cd fluxes in aspen's forest floor leachate were slightly but significantly higher than those in the other species' leachates (Fig 5.2). Nevertheless, metal leaching from the forest floor under aspen was not as high as foreseen, especially not when considering dissolved metal fluxes. This might imply that the risks of below-ground metal dispersion under aspen might actually be smaller than expected. More research on this issue is done in Chapter 7.

Aspen revealed elevated Cd fluxes on the one hand, but low H^+ fluxes and high base cation fluxes on the other hand in its forest floor leachate. The first process implies a risk of below-ground metal dispersion in the soil profile, whereas the latter processes might contribute to stabilization of the released metals in the soil. Given the current set-up, it is hard to determine which process will be decisive with respect to Cd and Zn leaching. Anyhow, until further research, the elevated metal concentrations in aspen's leaf litter make this species inappropriate

for phytostabilization purposes because of the risk of above-ground metal dispersion. Further research is thus essential, in particular on the chemical composition of the soil solution in deeper soil layers under the different tree species (see Chapter 7).

6

Can shrub species with higher litter quality mitigate soil acidification in pine and oak forests on poor sandy soils?

Abstract

This study, aiming to unravel whether topsoil conditions under tree species with nutrient-poor leaf litter can be altered by admixing a shrub layer, was performed in 12 pine (mainly *Pinus sylvestris*) and 12 oak (*Quercus robur*) stands on sandy podzolic soils in northeast Belgium. We examined the effects of presence of a shrub layer on forest floor mass and topsoil chemical properties related to soil acidification. The shrub species included were European rowan (*Sorbus aucuparia*), alder buckthorn (*Rhamnus frangula*) and black cherry (*Prunus serotina*). For each tree species, 60 – 90 years old stands were selected containing shrubs present in varying cover classes: sparse (< 20 %), intermediate (20 - 70 %) and dense (> 70 %). The oak stands were characterized by less but 'nutrient-richer' litterfall, compared to the pine stands. This was reflected in less humus build-up (FH-horizon) and higher pH, CEC, BS and lower C/N in the topsoil in the oak stands compared to the pine stands. However, despite the fact that the shrubs produced litter with significantly higher base cation and N concentrations than that of the studied tree species, we did not find any significant changes in topsoil conditions in the pine and oak stands under study, even under dense shrub layers (87 – 91 % cover).

After: Van Nevel, L., Mertens, J., De Schrijver, A., De Neve, S., Verheyen, K., 2014. Can shrub species with higher litter quality mitigate soil acidification in pine and oak forests on poor sandy soils? Forest Ecology and Management, 330, 38-45.

6.1 Introduction

Acidification of forest soils has been, and still is, an important concern in large parts of Europe and North America. Many studies have reported soil pH decreases in forests during the past decades (e.g. Johnston et al., 1986; Ahokas, 1997; Drohan and Sharpe, 1997; Hovmand and Bille-Hansen, 1999; Miller et al., 2001; Jönsson et al., 2003; De Schrijver et al., 2006). Atmospheric deposition of acidifying (SO_x, NO_y) and potentially acidifying (NH_x) compounds undoubtedly and unequivocally drives forest soils towards more acidic conditions, but the rate of soil acidification is also determined by the litter quality and the related build-up of the forest floor (De Schrijver et al., 2012). Sandy podzolic soils, which are naturally acidic and depleted in nutrients, are characterized by low cation exchange capacity (CEC) and low acid neutralizing capacity (Andersen et al., 2002) and have shown to be particularly susceptible to further acidification (Wiklander and Andersson, 1972). Such sandy podzolic soils are widespread in the northeastern part of Belgium, the region of the present study. On top of that, tree species choice is limited on these poor sandy soils, mainly coming down to species with nutrient-poor leaf litter, e.g. pine (*Pinus* spp.) and oak (*Quercus* spp.).

Soil acidification leads to numerous adverse ecological and biogeochemical effects on (forest) ecosystems, such as depletion of essential base cations (Mg^{2+}, Ca^{2+}, K^{+}) and increased availability of potentially toxic elements (e.g. Al^{3+}) (Bowman et al., 2008; Marlow and Peart, 2014). In this respect, we aimed to investigate whether mitigation of soil acidification is feasible by mixing pine and oak with shrub species that have higher nutritional leaf litter quality, potentially facilitating litter decomposition and base cation release to soils. In this study, leaf litter quality comprises base cation and N concentrations. After all, higher base cation concentrations and lower C/N ratio enhance litter decomposition (e.g. Hobbie et al., 2006; Zhang et al., 2008), promoting faster base cation cycling (Melillo et al., 1982), which will in turn increase the buffering capacity of the soil (De Schrijver et al., 2004; Clarholm and Skyllberg, 2013). Hence, in what follows, we will use the terms 'nutrient-rich' and 'nutrient-poor' leaf litter.

The ameliorative effects of European rowan (*Sorbus aucuparia*), alder buckthorn (*Rhamnus frangula*) and black cherry (*Prunus serotina*) have been proven during the last decades (e.g. Emmer et al., 1998; Kooijman et al., 2000; Starfinger et al., 2003; Lorenz et al., 2004; Becker et al., 2013; Carnol and Bazgir, 2013; Kacálek et al., 2013). The majority of these studies, however, compared species effects in homogeneous stands. However, tree/shrub species may have different effects when present in a mixture compared to pure stands (Vandermeer, 1989; Hättenschwiler et al., 2005). We found some studies though on the effects of shrub admixtures in stands composed of tree

species with nutrient-poor leaf litter (Brandtberg et al., 2000; Mohr and Topp, 2005; Aubert et al., 2006), but they dealt with other species and soil types than those we consider in the present study. Moreover, the required shrub cover to achieve a biogeochemical effect in the (top)soil is still underexplored. Hence, our key questions were (1) can soil acidification of podzol soils in pine and oak stands be counteracted by admixing shrubs such as *S. aucuparia*, *R. frangula* or *P. serotina* and (2) if so, what is the threshold cover of the shrubs needed to obtain a significant effect in the topsoil? We expect a species effect on the nutrient return especially via foliar litterfall (Prescott, 2002; Reimann et al., 2007; Vesterdal et al., 2008; Hojjati et al., 2009; Carnol and Bazgir, 2013). Therefore, we hypothezise in zones with dense shrub cover (i) a higher nutrient return to forest floor via litterfall, (ii) a thinner forest floor and (iii) a higher topsoil pH and base saturation, compared to zones without shrubs.

6.2 Materials and Methods

6.2.1 Study area and stand selection

The study was conducted in the Campine ecoregion, situated in the northeastern part of Belgium. The climate is sub-atlantic: the mean annual precipitation is about 800 mm and is evenly distributed throughout the year. The mean annual temperature is 9.0 °C (Royal Meteorological Institute of Belgium, http://www.kmi.be/). The region's characteristic forests are secondary pine plantations - mainly of Scots pine (*Pinus sylvestris*) and, to a lesser extent, Corsican pine (*P. nigra* subsp. *Laricio*) - on nutrient-poor and acidic sandy soils. The pine stands are interspersed with deciduous stands of pedunculate oak (*Quercus robur*), red oak (*Q. rubra*), common beech (*Fagus sylvatica*), silver birch (*Betula pendula*), and downy birch (*B. pubescens*). The most common species in the shrub layer are rowan (*S. aucuparia*), black cherry (*P. serotina*), and alder buckthorn (*R. frangula*) (Waterinckx and Roelandt, 2001).

We selected 24 stands (12 pine, 12 oak), spread over 9 forests on sandy soils in the Campine region. The distance between the paired oak and pine stands ranged from 100 to 1200 m. Only 60 – 90 years old stands, with a homogeneous, even-aged tree layer of pine (7 stands with *P. sylvestris* and 5 stands with *P. nigra*) or oak (*Q. robur*) and a tree canopy cover of more than 50% were considered for our study. Another crucial selection criterion was the presence of a shrub layer of European rowan (*S. aucuparia*), alder buckthorn (*R. frangula*) or black cherry (*P. serotina)*, with at least three cover classes present in each stand: sparse (< 20 %), intermediate (20 - 70 %), dense (> 70 %). In about half of the selected stands there was a homogeneous shrub layer of one of the three considered shrub species, whereas in the other half the shrub layer was dominated by

one of the considered species and blended with a minor share of one or two of the remaining shrub species. The shrubs had developed naturally in the stands (they were not planted).

Fig 6.1 shows the location of the selected forests in the Campine region. In these forests, the 24 stands under study were selected. The exact location, species composition, canopy cover and basal area of the tree and shrub layer of the 24 selected stands are presented in Table 6.1.

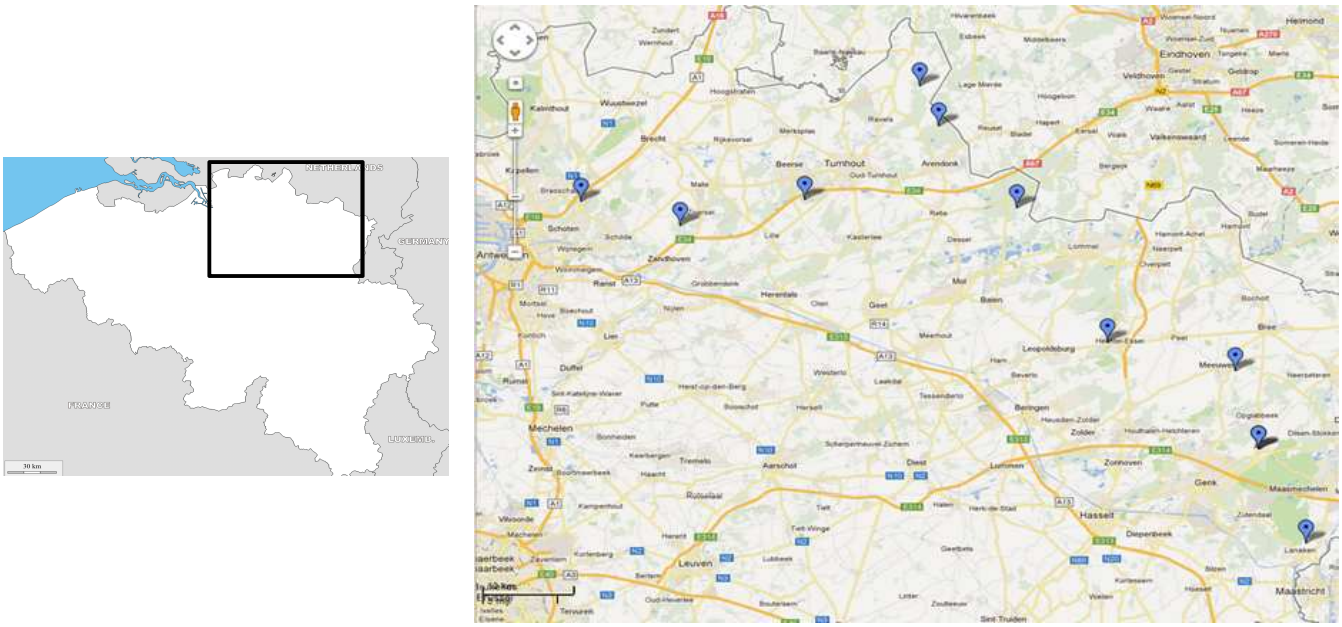


Figure 6.1 Map of the Campine region, showing the location of the forests in which the 24 stands were selected

6.2.2 Experimental set-up and sampling

In each of the 24 selected stands, the percentage cover of the tree and shrub layer was estimated visually in August 2011 and three shrub cover classes (sparse (S), intermediate (I), dense (D)) were allocated. Each cover class represented a patch within the stand of at least 20 m diameter. The center of each cover class was allocated as a sampling point. Around these sampling points, the basal area of trees and shrubs was measured and litterfall, forest floor and mineral soil were sampled as described below.

The basal area of all trees and shrubs with a diameter at 1.3 m height (DBH) > 7 cm was determined in a circle with radius 9 m around the sampling point, whereas the shrubs with DBH < 7 cm were measured within a smaller radius of 4.5 m.

Litterfall was collected with 1 m high litterfall traps that had a circular surface area of 0.24 m². Every sampling point was provided with a litterfall trap, which was installed in September 2011 and emptied monthly during autumn 2011 until the broadleaved species were leafless (October –

December). Litter samples were oven-dried (70 °C) to constant weight. Prior to weighing (± 0.01 g), samples were sorted out by hand to separate non-foliar litterfall (mainly twigs and fruits) and foliar litterfall of the considered species. As coniferous litter is shed year-round, annual litterfall amounts of the pine trees were obtained by proportionally upscaling their litterfall gained during autumn 2011. Therefore we used a conversion factor based on litterfall data of Scots pine, which was collected in 2007-2008 in Waaltjesbos (see § 3.2.2 and § 3.2.3). On the basis of this 2007-2008 dataset, we established that Scots pine clearly had a litterfall peak during autumn (October - December), representing 57% of its annual litterfall.

The forest floor was defined as the organic material above the mineral soil and was sampled in September - October 2012, just before the onset of (deciduous) foliar litterfall, when forest floor mass was at a minimum. The forest floor (comprising the litter (L), fragmentation (F) and humus (H) horizons (Jabiol et al., 1995)) was collected in two replicate squares within a radius of 1 m around each sampling point, using a 20 cm x 20 cm wooden frame. The three horizons (L, F, H) were collected separately. Sampling was done carefully in order to avoid contamination with the mineral material. Forest floor samples were oven-dried (70 °C) to constant weight; twigs, fruits and non-litter material were discarded and the remaining fraction was weighed (± 0.001 g).

Mineral soil sampling was carried out simultaneously with forest floor sampling. Underneath every removed forest floor sample, the topsoil (0 - 5 cm) was sampled with a Kopecky ring (diameter 5 cm), by pooling two core samples. Soil sampling was confined to the upper 5 cm of the soil, as changes in soil pH and buffering capacity were expected to occur here first (De Schrijver et al., 2012).

Sampling of the forest floor and the mineral topsoil

6.2.3 Sample analysis

Foliar litterfall samples were ground using a centrifugal mill (Retsch ZM1, Germany) and analyzed for Ca, K, Mg, C and N. The non-foliar litterfall fraction was not analyzed. K, Ca, Mg concentrations were measured by means of flame atomic absorption spectrophotometry (Varian SpectrAA-240) after a destruction in HNO_3–$HClO_4$. C and N concentrations were determined by elemental analysis (Vario Max CNS, Germany). Mineral soil samples were oven-dried to constant weight at 40 °C. Aggregates were broken and soil was passed through a 2 mm sieve. Soil pH-H_2O was determined in a 1:5 soil/water solution with a glass electrode. A subset of 41 soil samples was also analyzed for effective CEC (CEC_e), C and N. Effective CEC was calculated as the sum of K^+ + Mg^{2+} + Ca^{2+} + Al^{3+}, measured by flame atomic absorption spectrophotometry using $BaCl_2$ (0.1 M) as extractant (ISO 11260). C and N concentrations were determined by dry combustion (Vario Max CNS, Germany).

6.2.4 Data analysis

Base cation concentrations in foliar litterfall were calculated by summing the equivalents of K^+, Ca^{2+} and Mg^{2+}. Nutrient (base cation and N) return via litterfall was calculated by multiplying foliar litterfall amount by its nutrient concentrations. Base saturation of the topsoil was calculated as (K^+ + Mg^{2+} + Ca^{2+})/CEC_e. The values of forest floor mass and topsoil chemical properties of the two replicates per sampling point were pooled and their averages were used for further statistical analyses. As no H-horizon was developed in about half of the sampling points, we pooled the F and the H horizons and considered them together as FH-horizon in all further analyses.

The three shrub species were considered together as 'shrubs' in all further analyses, in order to perform more straightforward data analysis. Their litter quality (C/N ratio, base cation and N concentrations) is shown in Table 6.2. When more than one of the three shrub species was present in the shrub layer around a sampling point, their foliar litterfall and nutrient amounts were summed (Fig 6.2 a, e & f) and their nutrient concentrations and C/N ratio were calculated as weighted average, based on their respective foliar litterfall amounts (Fig 6.2 b-d).

To unravel whether establishment of the shrubs was related to tree density, correlations between basal area and foliar litterfall amounts of the shrubs on the one hand and basal area of the trees on the other hand were calculated by means of Pearson correlation coefficients.

Response variables were foliar litterfall and its nutritional content (C/N ratio, base cation and N concentrations and amounts), together with the forest floor mass (L- and FH-horizon) and

topsoil properties (pH-H_2O, CEC_e, base saturation, C concentration, C/N ratio). The experimental design was hierarchical, with shrub cover classes nested within tree species (stand type) and tree species replicated at forest level, so multilevel models were applied. The lme function in the nlme library of R version 3.0.1 was used here (Bates et al., 2011; R Core Team, 2014). First, we examined whether there was a tree species and/or a shrub cover effect on the response variables. Therefore, we constructed a model with 'forest' as random effect term and 'tree species' and 'shrub cover' (nested within tree species) as fixed effect terms. Subsequently, we split the dataset in two subsets - one with *Pinus* stands and another with *Quercus* stands - to examine the effect of the shrub layer within each stand type. Therefore, we built an analogous model with 'forest' as random effect term and only 'shrub cover' as fixed effect term, which was applied to the *Pinus* and *Quercus* datasets separately. Foliar litterfall amount was included as covariate in the models (those applied to forest floor mass and topsoil properties) to control for potential effects of differences in litter input on the forest floor and topsoil properties. But as it did not have a significant effect on any of the response variables, it was removed again. The effects of tree species (on the whole dataset) and of shrub cover (within each stand type) on the response variables were tested using analysis of variance (ANOVA).

Table 6.2 Foliar litterfall quality of the shrub species under study (average ± st.dev); differences between species were tested using analysis of variance (ANOVA) with a Bonferroni post hoc test, values with the same letter did not differ between species ($p < 0.05$)

	BC (meq kg^{-1})	N (%)	C/N (-)
alder buckthorn	1164 ± 140 [b]	2.16 ± 0.25 [b]	23.5 ± 3.1 [a]
European rowan	995 ± 156 [a]	2.06 ± 0.25 [b]	25.2 ± 3.2 [a]
black cherry	944 ± 193 [a]	1.52 ± 0.37 [a]	35.4 ± 10.1 [b]

Table 6.1 Location, species composition, canopy cover and basal area of the tree and shrub layer (in cover classes S, I, D) of the selected stands

Forest	Location		Tree species	Tree cover [1] (%)	Basal area trees S - I - D [§] (m^2 ha^{-1})	Main shrub species	Shrub cover S - I - D [§] (%)	Basal area shrubs S - I - D [§] (m^2 ha^{-1})
Peerdsbos	Brasschaat	51°16' N	*Pinus*	55	31.8 - 25.6 - 29.3	*S. aucuparia*	20 - 60 - 80	2.9 - 4.8 - 6.4
		4°29' E	*Quercus*	85	19.3 - 17.5 - 36.3	*S. aucuparia*	5 - 35 - 90	0 - 4.0 - 7.8
Zoerselbos	Zoersel	51°16' N	*Pinus*	75	36.3 - 31.7 - 44.6	*S. aucuparia*	0 - 45 - 90	0 - 1.0 - 2.2
		4°42' E	*Pinus*	70	40.4 - 42.5 - 30.5	*R. frangula*	2 - 50 - 95	0.3 - 2.9 - 7.0
			Quercus	60	30.5 - 31.9 - 16.6	*R. frangula*	5 - 60 - 95	0.6 - 6.3 - 5.0
			Quercus	80	29.5 - 28.1 - 17.9	*S. aucuparia*	10 - 60 - 85	0.6 - 3.8 - 6.4
Grotenhout	Vosselaar	51°18' N	*Pinus*	60	22.4 - 43.8 - 36.1	*R. frangula*	0 - 45 - 90	0 - 1.0 - 2.1
		4°53' E	*Pinus*	55	14.8 - 24.9 - 40.8	*R. frangula*	2 - 60 - 95	0 - 3.8 - 3.9
			Quercus	85	10.1 - 23.3 - 11.8	*S. aucuparia*	10 - 45 - 90	0.2 - 2.6 - 11.2
			Quercus	75	12.1 - 25.8 - 18.2	*Sorbus & Rhamnus*	0 - 55 - 80	0.2 - 7.5 - 7.4
Overheide	Ravels	51°24' N	*Pinus*	70	28.2 - 28.5 - 44.6	*R. frangula*	0 - 40 - 100	0 - 0.6 - 3.0
		4°59' E	*Quercus*	80	19.0 - 21.6 - 19.8	*R. frangula*	10 - 60 - 100	0 - 1.3 - 7.5
Hoge Vijvers	Arendonk	51°21' N	*Pinus*	75	44.0 - 51.0 - 42.0	*R. frangula*	0 - 45 - 95	0 - 0.2 - 2.2
		5°05' E	*Quercus*	75	16.7 - 9.5 - 10.4	*R. frangula*	0 - 50 - 100	0.3 - 1.5 - 5.4
Gruitrodebos	Meeuwen-Gruitrode	51°04' N	*Pinus*	55	44.9 - 24.8 - 14.5	*P. serotina*	5 - 65 - 90	0.3 - 2.6 - 2.9
		5°36' E	*Pinus*	75	55.2 - 45.8 - 43.8	*P. serotina*	1 - 62 - 95	0 - 2.4 - 3.6
			Quercus	65 (80)	22.4 - 28.2 - 6.9	*R. frangula*	7 - 40 - 70	0 - 0.5 - 1.6
			Quercus	70 (90)	21.4 - 28.4 - 21.8	*P. serotina*	8 - 45 - 75	1.3 - 0.8 - 3.7
In Den Brand	Hechtel-Eksel	51°08' N	*Pinus*	85	48.8 - 50.9 - 44.1	*R. frangula*	7 - 40 - 77	0 - 0.9 - 1.2
		5°20' E	*Quercus*	85 (95)	21.9 - 23.6 - 20.9	*R. frangula*	10 - 50 - 90	0.1 - 0.4 - 2.1
Pietersembos	Lanaken	50°54' N	*Pinus*	60	25.0 - 31.6 - 40.0	*P. serotina*	15 - 53 - 92	0 - 5.5 - 4.8
		5°37' E	*Quercus*	75 (90)	26.5 - 20.9 - 32.3	*R. frangula*	0 - 65 - 80	0 - 1.6 - 1.3

Postel	Postel	51°17' N 5°11' E	*Pinus* *Quercus*	85 65 (80)	25.6 - 30.7 - 43.3 32.3 - 13.7 - 21.3	*P. serotina* *P. serotina*	0 - 62 - 95 20 - 55 - 85	0 - 1.5 - 5.9 0 - 2.2 - 3.8
		Averages	*Pinus* *Quercus*	68 75 (81)	34.8 - 35.8 - 37.8 21.5 - 21.0 - 18.2		4 - 52 - 91 7 - 52 - 87	0.3 - 2.3 - 3.8 0.3 - 2.7 - 5.3

[1] Tree cover of the main tree species (pine or oak); values between brackets denote total tree cover (main + additional tree species)

[§] S=sparse, I=intermediate, D=dense

6.3 Results

6.3.1 Foliar litterfall

Annual foliar litter production from the pine trees was higher compared to the oak trees (Table 6.3). Total foliar litterfall (trees + shrubs) was also higher in the pine stands than in the oak stands (Table 6.4). Relative contributions of shrub litterfall to total litterfall amounted to 0.1%, 6% and 17% on average, in the sparse, intermediate and dense shrub cover classes, respectively (Fig 6.2a). Within the pine stands, there was a significant effect of shrub cover, with higher total litterfall amounts in the zones with a denser shrub layer. However, despite similar relative contributions of shrub litterfall to total litterfall in both stand types, this effect could not be found in the oak stands (Table 6.4 and Fig 6.2a). Correlation analyses revealed that neither basal area, nor foliar litterfall of the shrubs was related to the basal area of the trees (data not shown).

Litterfall quality was significantly better in the oak stands compared to the pine stands, as was shown by lower C/N ratio and higher base cation and N concentrations (Table 6.4 and Fig 6.2 b-d). Furthermore, the shrubs produced higher quality litter than the tree species (Table 6.3). The species effect was particularly remarkable for base cation concentrations, being 3.5 times higher in the shrub litterfall compared to the pine litterfall, and almost double compared to the oak litterfall. Hence, the denser the shrub layer, the higher the base cation concentrations were, both in the pine and oak stands (Table 6.4). However, for N, this pattern could only be retrieved in the pine stands, and the C/N ratio was not influenced by the shrub cover (Table 6.4).

Even though there was less foliar litterfall in the oak stands (Table 6.4 and Fig 6.2a), the base cation and N return in these stands was significantly higher compared to the pine stands. Nevertheless, the presence of a dense shrub layer in the pine stands induced significantly higher nutrient amounts via litterfall, reaching the level of those in the oak stands (Table 6.4 and Fig 6.2 e-f). No effects of shrub cover on nutrient return were found in the oak stands. Relative contributions of the shrubs' base cation amounts to total base cation return amounted to 27% and 40% on average, in the Q-D and P-D zones respectively (Fig 6.2e).

Table 6.3 Foliar litterfall and its nutrient concentrations and C/N ratio of the trees and shrubs under study (average ± st.dev); differences between species were tested using analysis of variance (ANOVA) with a Bonferroni post hoc test, values with the same letter did not differ between species ($p < 0.05$)

		Foliar litterfall ($kg\ ha^{-1}\ yr^{-1}$)		BC ($meq\ kg^{-1}$)	N (%)	C/N (-)
pine		2891 ± 581 [b]		300 ± 94 [a]	0.93 ± 0.16 [a]	59.1 ± 9.2 [c]
oak		2343 ± 678 [a]		574 ± 80 [b]	1.53 ± 0.17 [b]	34.0 ± 3.7 [b]
shrubs [§]	S	4 ± 11	P	1008 ± 223 [c]	1.90 ± 0.41 [c]	28.7 ± 9.3 [a]
	I	158 ± 123	Q	1113 ± 125 [d]	2.03 ± 0.36 [c]	25.5 ± 5.3 [a]
	D	500 ± 264				

[§] Shrubs' foliar litterfall amounts in cover classes S, I, D (S=sparse, I=intermediate, D=dense) and their litter quality in the pine (P) and oak (Q) stands

6.3.2 Forest floor mass

Forest floor mass was significantly affected by the tree species: the mass of the L-horizon was higher in the oak stands, whereas the FH-horizon mass was higher in the pine stands (Table 6.4).

Within the pine stands, there was a significant effect of shrub cover on the L-horizon mass, with lowest mass in the zones with sparse shrub cover. No effect of shrub cover on the mass of other forest floor horizons (FH pine, L & FH oak) could be found (Table 6.4).

6.3.3 Topsoil

pH, CEC_e as well as base saturation of the topsoil were significantly higher in the oak stands than in the pine stands, whereas C/N ratio was significantly lower. There was no tree species effect on the C concentration. The presence of a shrub layer did not have significant effects on topsoil properties (Table 6.4).

Table 6.4 Total foliar litterfall (trees + shrubs) quantity and quality, forest floor mass and topsoil properties (average ± st.dev) in the pine and oak stands, and under the 3 shrub cover classes (S, I, D) within the respective stands (n = 72). p-values denote the effects of (1) tree species and (2) shrub cover within each tree species on the response variables, tested using analysis of variance (ANOVA) within linear mixed-effect models[1,2]. See methods for details on the model building.

	Tree species			*Pinus*				*Quercus*			
	Pinus	*Quercus*	*p* [1]	S §	I §	D §	*p* [2]	S §	I §	D §	*p* [2]
Foliar litterfall											
Amount (kg/ha/yr)	3195 ± 633	2553 ± 626	*< .0001*	2837 ± 537	3160 ± 546	3589 ± 613	*0.001*	2486 ± 772	2605 ± 605	2569 ± 524	*0.82*
C/N (-)	56.9 ± 9.4	33.8 ± 4.0	*< .0001*	58.9 ± 9.3	58.3 ± 9.5	53.6 ± 9.4	*0.13*	33.8 ± 4.5	34.8 ± 3.7	32.6 ± 3.8	*0.09*
BC conc (meq/kg)	340 ± 106	615 ± 94	*< .0001*	281 ± 95	330 ± 111	408 ± 76	*0.002*	571 ± 58	613 ± 105	663 ± 96	*0.02*
N conc (%)	1.01 ± 0.18	1.56 ± 0.19	*< .0001*	0.93 ± 0.17	0.98 ± 0.15	1.11 ± 0.19	*0.004*	1.55 ± 0.23	1.51 ± 0.15	1.63 ± 0.16	*0.07*
BC return (eq/ha/yr)	1084 ± 402	1556 ± 411	*< .0001*	792 ± 311	1023 ± 362	1437 ± 231	*0.0001*	1416 ± 463	1559 ± 337	1693 ± 408	*0.17*
N return (kg/ha/yr)	32.4 ± 10.8	39.6 ± 10.1	*0.0002*	26.6 ± 7.4	30.5 ± 5.6	40.2 ± 13.4	*< .0001*	38.1 ± 11.5	38.7 ± 8.1	41.9 ± 11.0	*0.46*
Forest floor mass											
L (ton/ha)	3.4 ± 1.8	4.1 ± 1.2	*0.04*	2.6 ± 1.6	3.9 ± 1.9	3.9 ± 1.6	*0.02*	4.3 ± 1.1	3.8 ± 1.0	4.2 ± 1.3	*0.46*
FH (ton/ha)	54.5 ± 28.7	37.9 ± 24.3	*0.004*	48.0 ± 28.0	57.4 ± 27.5	58.1 ± 31.8	*0.39*	40.4 ± 28.1	39.5 ± 26.3	33.7 ± 19.2	*0.77*

Topsoil										
pH-H2O (-)	3.92 ± 0.15	4.03 ± 0.23	*0.01*	3.95 ± 0.19	3.90 ± 0.12	3.90 ± 0.14	*0.59*	4.01 ± 0.26	4.05 ± 0.22	4.02 ± 0.24	*0.86*
CEC_e * (cmol/kg)	2.2 ± 0.7	4.4 ± 1.9	*0.004*	2.0 ± 0.4	1.8 ± 0.5	2.6 ± 0.8	*0.16*	4.0 ± 2.7	4.8 ± 2.0	4.4 ± 1.2	*0.86*
BS * (%)	28.6 ± 10.4	47.0 ± 15.9	*0.006*	28.4 ± 11.7	24.0 ± 8.2	32.1 ± 12.0	*0.68*	46.6 ± 18.2	39.3 ± 5.3	55.2 ± 19.8	*0.30*
C * (%)	5.4 ± 2.6	7.6 ± 4.1	*0.16*	3.8 ± 1.0	5.7 ± 3.2	6.7 ± 2.8	*0.20*	7.7 ± 6.9	6.9 ± 2.7	8.2 ± 2.4	*0.91*
C/N * (-)	19.2 ± 2.7	15.8 ± 3.2	*0.01*	18.4 ± 3.5	20.1 ± 3.3	19.4 ± 2.1	*0.74*	17.1 ± 4.3	15.0 ± 3.4	15.3 ± 1.7	*0.40*

* analyzed on a subset of samples (n = 23)

§ S=sparse, I=intermediate, D=dense

Full factorial model R syntaxes:

[1] Response ~ tree species + tree species:shrub cover + (1 | forest)

[2] Response ~ shrub cover + (1 | forest)

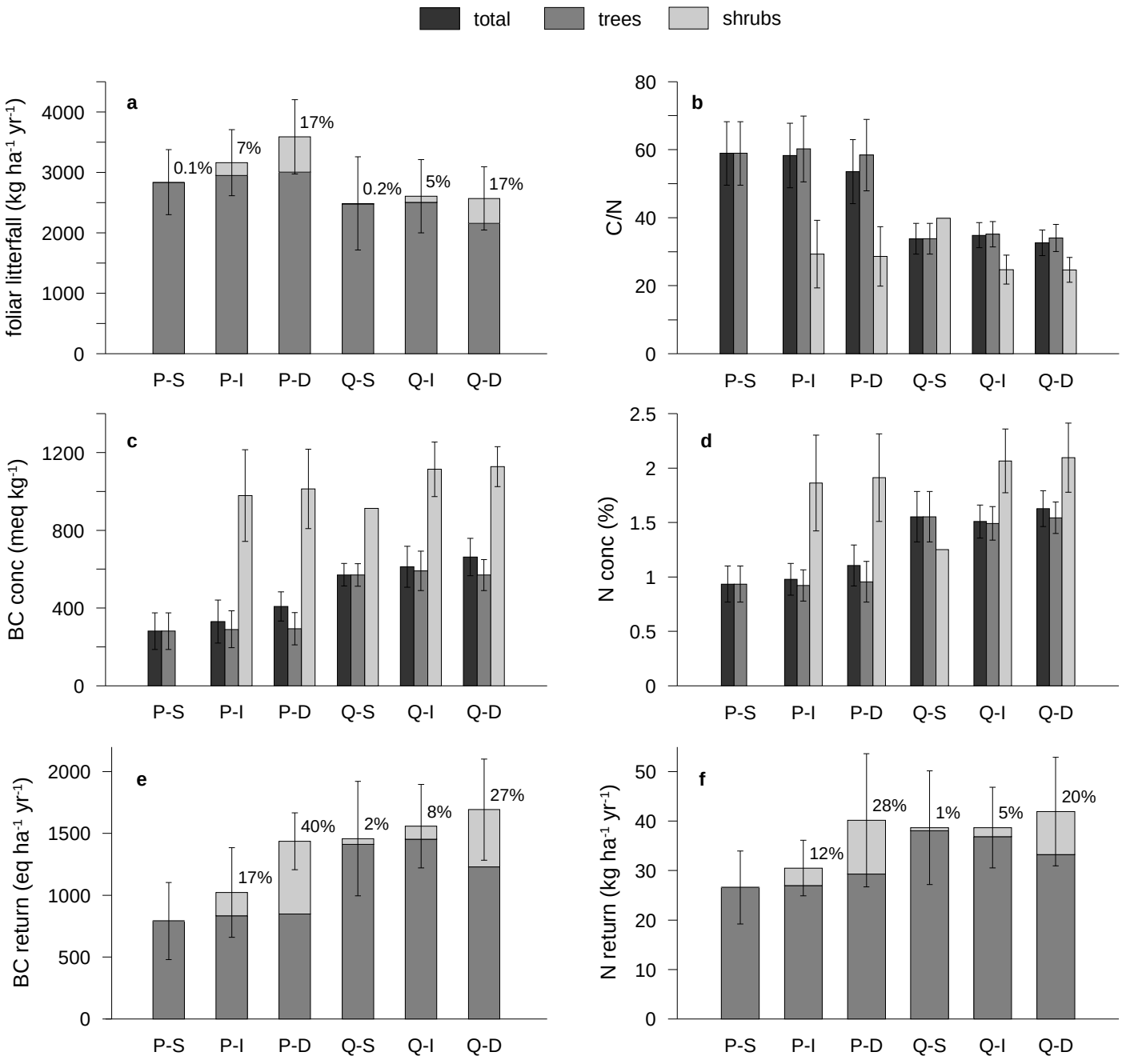


Figure 6.2 Foliar litterfall quantity and quality under the 3 shrub cover classes (S, I, D) within the pine (P) and oak (Q) stands: foliar litterfall amounts (a), C/N ratio (b), base cation and N concentrations (c-d) and amounts (e-f) (average ± st.dev). Percentages (a, e-f) denote relative contributions of shrubs' to total amounts.

6.4 Discussion

6.4.1 Foliar litterfall

Litterfall amount and quality affect the magnitude of the nutrient return from the forest canopy to the soil. Foliar litter production from the tree species (Table 6.3) was in line with similar studies on pine (Maddelein and Lust, 1992) and oak (Hansen et al., 2009), but higher than the values reported by Pausas (1997) and Reich et al. (2005).

The presence of a shrub layer resulted in increased litterfall amounts in the pine stands, yet this was not the case in the oak stands, notwithstanding similar contributions of shrub litterfall to total litterfall in both stand types (Table 6.4 and Fig 6.2a). The pattern in the oak stands corresponded to what one could expect: a denser shrub layer in zones where the tree layer was sparser, thus yielding an equal amount of litterfall in zones with or without shrubs. However, as there was no correlation between basal area or litterfall of the shrubs and basal area of the trees, it seems that the establishment of the shrubs in the stands under study took place independently of tree density. The fact that the stands were quite open might explain this.

There was a significant species effect on the nutrient concentrations and C/N ratio in foliar litterfall, resulting in a decreasing quality in the order shrubs > oak > pine (Table 6.3). Analogous results were found in other studies considering the same species (Lorenz et al., 2004; Reich et al., 2005; Hobbie et al., 2006; Carnol and Bazgir, 2013; Kacálek et al., 2013). Base cation concentration in the shrubs' foliar litterfall was higher in the oak stands than in the pine stands (Table 6.3), probably induced by a higher topsoil CEC_e in the oak stands (Table 6.4). Litterfall quality (nutrient concentrations and C/N ratio) in the oak stands, even without shrubs, was higher than in the pine stands with dense shrub layers (Table 6.4 and Fig 6.2 b-d). This implies that converting pine stands towards oak stands would be more effective to generate nutrient-richer litterfall than planting shrubs under the pines.

The shrubs' contribution to the total base cation return in the Q-D (27%) and P-D (40%) zones (Fig 6.2e) was strikingly high given the low contribution of the shrubs to the total basal area (Table 6.1) and to the litterfall amounts (Fig 6.2a). This illustrates the relative importance of a shrub layer with species that have higher nutritional leaf litter quality. Nevertheless, the impact of increased nutrient return on the topsoil might as well be marginal. After all, litterfall quality will, more than litterfall quantity, influence litter decomposition and thus determine nutrient availability and cycling (Berg et al., 2001; Reich et al., 2005; Hansen et al., 2009). The fact that litterfall quality in the P-D zones was lower than in the oak stands (Table 6.4 and Fig 6.2 b-d), implied that the equal nutrient return was a matter of litterfall amounts. Therefore we expect that

oak stands without shrubs will still have better soil properties than pine stands with shrubs. Within the oak stands, shrubs did not have an impact on nutrient return via litterfall, due to a less pronounced difference between litterfall quality of oaks and shrubs (Table 6.3) on the one hand, and equal litterfall amounts in zones with or without shrubs (Table 6.4 and Fig 6.2a) on the other hand. Our hypothesis (i) of higher nutrient return in zones with a denser shrub layer was thus only supported in the pine stands.

6.4.2 Forest floor mass

Our data revealed a discrepancy between litterfall amounts and L-horizon mass: although litterfall was highest in the pine stands, the mass of their L-horizons was lowest (Table 6.4). This might imply that fragmentation of the litter layer under the pines proceeded faster than under the oaks. We suggest that this could be attributed to the leaf shape of both species. Firstly because the relative contact area between leaf tissue and decomposing organisms is higher for needles than for leaves, and secondly because a litter layer composed of needles has more direct contact with the underlying forest floor or soil than a litter layer composed of oak leaves (which is less dense). The former is thus better accessible to soil decomposers. Fast litter mass loss in the early decomposition stages has also been observed for Scots pine in previous studies (Hobbie et al., 2006; see also Chapter 4).

Once the litter is fragmented, leaf shapes become irrelevant and litter quality will be one of the dominant regulators of litter decomposition (Melillo et al., 1982; Berg et al., 1993; Gholz et al., 2000; Prescott et al., 2004; Hobbie et al., 2006; Cornwell et al., 2008; Zhang et al., 2008). Given the inferior quality of the pine litter (Table 6.3), the higher mass of FH-horizons in the pine stands (Table 6.4) was thus in line with the expectations.

Shrub cover hardly affected the forest floor mass in both stand types. Only the L-horizon mass in the pine stands was significantly higher when a shrub layer was present (Table 6.4). As we suggested earlier, the fragmentation of the litter layer is probably highly directed by the shape of the leaves/needles, developing faster for needle litter than for leaf litter. Our results, showing lowest L-mass in zones without shrubs under the pines, were consistent with this reasoning. Also the fact that shrub litterfall did not have an effect on the fragmentation process (expressed as L-horizon mass) in the oak stands, agreed with our assumption.

However, the lack of impact of shrub cover on the mass of the FH-horizons (Table 6.4) was rather unexpected, given the fact that the development of FH-horizons is governed by litter quality (Cornwell et al., 2008; Zhang et al., 2008). After all, the higher quality of the shrub litter

(Table 6.3) was reflected in higher base cation concentrations (in both pine and oak stands) and N concentrations (only in pine stands) in litterfall in zones with more shrubs (Table 6.4). Therefore, we presumed that zones with a denser shrub layer would be characterized by thinner FH-horizons, due to faster decomposition. But our hypothesis (ii) was thus not supported. We even found a contrary (although not significant) trend in the pine stands, suggesting higher FH-horizon mass in denser shrub zones. This was most probably caused by the considerably higher litter input in denser shrub zones under the pines (Table 6.4 and Fig 6.2a). However, as the litterfall quality in these zones was higher as well (Table 6.4), we assume that the litter decomposed faster, ensuring the significant surplus of litterfall amounts in these zones not to result in a significant surplus of FH-horizon mass. The oak stands, on the other hand, produced an equal amount of litterfall in zones with or without shrubs, but with higher base cation concentrations in the denser shrub zones (Table 6.4). Hence, due to the latter, slightly faster litter decomposition and thus thinner FH-horizons were expected in these dense shrub zones. There was indeed a trend towards thinner FH-horizons in the Q-D zones, yet this was not significant, probably because the shrubs in the oak stands did not affect litterfall N concentration and C/N ratio (Table 6.4), both known as essential drivers of litter decomposition (e.g. Edmonds, 1980; Berg et al., 1993; Aerts, 1997; Jamaludheen and Kumar, 1999; Moro and Domingo, 2000; Zhang et al., 2008).

The lack of significant impact of shrub cover on FH-horizons in the oak and pine stands might indicate a threshold effect. After all, both in P-D and Q-D zones, the shrubs constituted only 17% of the total litterfall (Fig 6.2a). A higher contribution of shrub litterfall to total litterfall would increase the litter quality, by which the effects on the FH-horizons would probably become more pronounced. However, as the shrub cover was already very high in the dense shrub zones (on average 91% and 87% in the P-D and Q-D stands respectively; cfr. Table 6.1), much higher shrub litterfall amounts were not very likely, implying that the threshold could not be attained.

6.4.3 Topsoil

The topsoil in the oak stands had significantly higher pH, CEC_e and BS and lower C/N ratio than in the pine stands (Table 6.4). This was most probably the result of higher litterfall quality (in terms of C/N ratio and base cation concentration) and less humus build-up (FH-horizon) in the oak stands compared to the pine stands (Table 6.4). De Schrijver et al. (2012) also showed that forest floor build-up and leaf litter quality were the determining factors for changes in the topsoil pH, exchangeable Ca^{2+} and Al^{3+} concentrations. Additionally, it is generally known that

conifers receive higher dry deposition of acidifying compounds (SO_4^{2-}, NH_4^+, NO_3^-) than deciduous trees (Augusto et al., 2002; Erisman and Draaijers, 2003; De Schrijver et al., 2004, 2007, 2008; Wuyts et al., 2008), which probably intensified the difference in topsoil conditions between the oak and the pine stands. Finally, local differences in land use history in the selected forests might also have contributed to this pattern.

The presence of shrubs did not have any significant effect on the topsoil properties though, neither under the oaks, nor under the pines (Table 6.4). Yet, in the pine stands the C concentrations in the P-D zones were almost twice as high compared to the P-S zones, linked to significantly higher litter input in denser shrub zones under the pines (Table 6.4 and Fig 6.2a). The fact that CEC_e, BS and C/N were not significantly affected by the shrubs in the oak stands was not surprising as the shrub layer did not even have an effect on the nutrient return via litterfall (Table 6.4), due to the minor difference between litterfall quality of oaks and shrubs (Table 6.3). However, considering the pine stands, we had expected that the higher nutrient concentrations in litterfall in denser shrub zones under the pines (Table 6.4) would have been reflected in improved topsoil conditions. After all, as we assumed that the nutrient-richer shrub litterfall enabled faster litter decomposition in the denser shrub zones under the pines (cfr. § 6.4.2), one would expect faster nutrient cycling in those zones, resulting in higher buffering capacity of the underlying (top)soil (in terms of CEC_e and BS). Yet this was not the case, so the question was where the surplus of base cations and N that reached the forest floor via litterfall in the denser shrub zones under the pines was gone? It was very likely that the nutrients were stored in the forest floor (FH-horizon), as was also found by several other authors (e.g. Ovington, 1958; Maddelein and Lust, 1992; Emmer et al., 1998; Fisher and Binkley, 2000). The pine stands in our study were indeed characterized by a relatively thick FH-horizon. Hence, the nutrients originating from the shrubs' richer litterfall might be 'trapped' in the forest floor and temporarily removed from the active nutrient cycles, as organic matter breakdown may take several years (Ovington, 1954). Another process that could explain why the buffering capacity of the topsoil (CEC_e and BS) was not significantly increased in the P-D zones, might be leaching of the nutrients from the topsoil.

Summarizing, there was a clear tree species/stand type effect on the topsoil properties, but no significant effects of shrub cover could be found. Our hypothesis (iii) was thus not supported. One might assume that more shrubs could have induced more effects in the topsoil (a threshold effect). However, given the high shrub cover in the dense shrub zones (Table 6.1), we believe that a higher shrub impact is not realistic (cfr. § 6.4.2). The age of the shrubs, on the other hand, might have been a reason for the lack of effects. The exact age of the shrubs was unknown, but

as they were well-developed we estimated that they were established in the stands since a couple of decennia. Following Emmer et al. (1998) and Vesterdal et al. (2002), we might expect chemical changes in the mineral (top)soil to emerge in the future. After all, 'deacidification' of forest soils is a slow and lengthy process (Vanguelova et al., 2010; Karlsson et al., 2011; Verstraeten et al., 2012; Akselsson et al., 2013), certainly given the high rates of acidifying dry deposition in industrialized regions (De Schrijver et al., 2012). On the other hand, it might be quite likely that litter quality was not effective enough to alter the soil quality on our nutrient-poor and acidic sandy soils, and that it never will. Hommel et al. (2007) indeed stated that litter quality was the key factor to restore the buffering capacity of forest topsoils, yet this mechanism was only successful within a range of 'intermediate' (moderately poor) sites. Sandy soils should e.g. contain at least 15% loam to be susceptible for the 'nutrient-rich litter effect' (Hommel et al., 2007).

Hence, our results suggest that planting shrub species with nutrient-richer litter in pine and oak stands will not change topsoil properties as soil acidity and base saturation, at least in the short to medium term. It seems that adequate tree species selection (or conversion of pine towards deciduous tree species) will probably be more effective to enhance topsoil conditions than planting shrubs under tree species with nutrient-poor leaf litter.

6.5 Conclusions

Despite the fact that the shrubs produced litter with significantly higher nutrient concentrations than that of the tree species, we did not find any effects on topsoil conditions in the pine and oak stands under study, even under dense shrub layers (87 – 91 % cover). Consequently, it seems thus not feasible to mitigate (top)soil acidification by introducing a shrub layer with nutrient-rich leaf litter in pine and oak stands on poor sandy soils, at least in the short to medium term. On the other hand, there was a tree species (stand type) effect, with less but 'richer' litterfall in the oak stands. This was reflected in faster litter decomposition (thinner FH-horizon) and higher pH, CEC_e, BS and lower C/N in the topsoil in the oak stands compared to the pine stands. Adequate tree species selection (or conversion of pine towards deciduous tree species) seems thus more effective to mitigate (top)soil acidification than planting shrubs under tree species with nutrient-poor leaf litter. However, given the poor and acidic conditions of the sandy podzolic soils in the Campine region, the impact of the tree species that can thrive on this soil type will most probably be insufficient to affect topsoil properties related to soil acidification thoroughly.

7

Water and element seepage fluxes under six different tree species on a metal contaminated site

7.1 Introduction

Tree cover provides a sustainable vegetation that may potentially allow metals to remain permanently stabilized in soil or woody biomass. Due to their high evapotranspiration capacity, trees might reduce downward soil water fluxes and metal leaching losses to the groundwater (Pulford and Watson, 2003). Garten (1999) modeled the effect of a forest cover on Sr leaching from contaminated soil, mainly in shallow subsurface flow, and showed that the losses were reduced by approximately 16% under trees relative to grass. Indeed, forest ecosystems are characterized by higher LAI, higher roughness length and deeper root systems compared to other vegetation types, resulting in a significantly higher evapotranspiration capacity (Kelliher et al., 1993; Zhang et al., 2001; Nosetto et al., 2005). However, evidence demonstrating that trees stabilize metals in the soil is still insufficient. It is known that metals can become vertically mobile in soil profiles under mature woodlands (Martin and Coughtrey, 1987; Dickinson et al., 1996; Clemente et al., 2008) and, clearly, this might threaten underground aquifers in the longer term (Pulford and Dickinson, 2005; Clemente et al., 2008). Other studies in highly contaminated mature woodlands, on the other hand, have demonstrated very low metal mobility and relatively steady-state conditions (Lepp and Dickinson, 2003). After all, transport of metals through the soil matrix is not only driven by evapotranspiration rates but also by a plethora of interrelated chemical reactions between the solid and the aqueous phase of the soil, including inorganic and organic complexation, oxidation-reduction reactions, precipitation-dissolution reactions and adsorption-desorption reactions (McLean and Bledsoe, 1992; Ross, 1994). Further research is thus required to evaluate the long-term feasibility of using trees to stabilize trace metals in soil.

Within a forest ecosystem, there is a tree species effect on evapotranspiration rates (Köstner, 2001; Zirlewagen and von Wilpert, 2001) as well as on leaching of organic and inorganic elements and ligands which affect metal solubility (e.g. Finzi et al., 1998; Augusto et al., 2002, 2014; De Schrijver et al., 2007, 2012; Fröberg et al., 2011). This implies that one of the key questions involved in realizing a sustainable and ecologically sound phytostabilization project is: which tree species will guarantee the lowest below-ground metal dispersion risks? Hence, the main goal of this study was to quantify the downward metal fluxes (seepage fluxes) under different tree species. Therefore, we collected the soil solution with suction cup lysimeters at 50 cm depth, for chemical analysis, and simulated the water fluxes at that depth by means of a numerical water balance model.

We hypothesize that (i) tree species which induce leaching of anions, base cations and/or protons will cause elevated Cd and Zn seepage fluxes and (ii) tree species with higher evapotranspiration rates will reduce Cd and Zn seepage fluxes.

7.2 Materials and Methods

7.2.1 Experimental set-up and sampling

The study included the six considered tree species (oak, silver birch, black locust, aspen, Scots pine, Douglas fir) and was executed in the selected stands in 'Waaltjesbos' (see Fig 3.1). See § 3.2.1 for full site descriptions and stand selection. A characterization of the soil of the study site is given in Table 3.5 (soil characteristics pH-KCl, OC content, CEC) and Table 3.6 (total and extractable soil Cd and Zn concentrations). The six tree species have divergent effects on the soil properties and the water balance and show different behaviour in metal cycling. Hence, seepage fluxes under these tree species are expected to reflect the biogeochemical and biophysical interactions that took place over the preceding years.

In each selected stand, two suction cup lysimeters were installed at 50 cm depth to collect soil solution. The lysimeters consisted of (i) a PVC tube fitted with a porous ceramic cup and (ii) an opaque, glass, one-liter bottle connected to the PVC tube via a polyethylene tube and stored below ground to keep samples cool. The lysimeters were installed into the soil at an angle of 45° and a -50 kPa suction was applied. Following installation, they were flushed during four months prior to the actual monitoring, which was executed three-weekly between December 2009 and November 2010. On each sampling occasion, sample volumes in the lysimeters were measured in the field, and 300-ml subsamples were taken to the lab for chemical analysis. The samples were transported in a cooler, once in the lab they were stored in a freezer until analysis. Simultaneously with soil solution monitoring, groundwater levels were monitored with 2 m long observation wells in five stands spread over the forest.

Suction cup lysimeter

7.2.2 Sample analysis

Soil solution samples from different sampling occasions were pooled volume-weighted to bulk samples per season (winter: 23/11/2009 – 31/03/2010; spring: 01/04/2010 – 25/06/2010; summer: 26/06/2010 – 21/09/2010; autumn: 22/09/2010 – 25/11/2010).

Prior to analyses, bulk samples were filtered through a 0.45 µm nylonfilter (Gelman), except for pH and total Cd and Zn concentrations, which were measured on the unfiltered samples. pH was measured with a pH Orion electrode; hydrogen ion (H^+) concentration was calculated from the measured pH value. Total Cd and Zn concentrations were measured with ICP-MS (Perkin-Elmer Elan DRC-e) and ICP-OES (Varian Vista-MPX) respectively, after a destruction with HNO_3 and H_2O_2. Filtered samples were analyzed for K^+, Na^+, Ca^{2+}, Mg^{2+}, Al^{3+} and NH_4^+ (flame atomic absorption spectrophotometry, Varian SpectrAA-240), for NO_3^-, SO_4^{2-}, PO_4^{3-} and Cl^- (ion chromatography, Dionex ICS-90), for DOC (Total Organic Carbon Analyzer, Shimadzu TOC-VCPN) and for dissolved Cd and Zn (respectively ICP-MS, Perkin-Elmer Elan DRC-e and ICP-OES, Varian Vista-MPX).

7.2.3 Soil water fluxes

Since soil water fluxes cannot be measured directly, they have to be estimated indirectly (Haines et al., 1982; Kutílek and Nielsen, 1994; Weihermuller et al., 2007; Nieminen, 2011). Soil water fluxes at 50 cm depth were estimated with the numerical Hydrus-1D model (Šimůnek et al., 1998). Soil physical characteristics [soil water retention curve, saturated hydraulic conductivity (K_{sat}) and volumetric soil water content (SWC)], precipitation and evapotranspiration rates are the driving variables in the model. For each of the considered tree species, one of the three selected stands was used to measure the soil physical characteristics. SWC was monitored by Time Domain Reflectometry (TDR) from March 2011 until February 2013. In every stand, four TDR probes (type CS625, Campbell Scientific) were installed (at 5, 15, 30 and 50 cm depth), and connected to a datalogger (type CR200, Campbell Scientific) which recorded SWC every 4 hours. Measurements of the soil water retention curve and K_{sat} were executed in the lab on undisturbed soil samples, taken with Kopecky rings in three replicates at 5, 15, 30 and 50 cm depth in September 2011. The samples' soil water retention curve was constructed by measuring SWC at nine soil-matric potentials (cfr. Cornelis et al., 2005). K_{sat} was obtained with the constant-head method, using a closed permeameter (Eijkelkamp Agrisearch Equipment) (cfr. Pulido Moncada et al., 2014).

TDR probes at 5, 15, 30 and 50 cm depth (left) and the tipping bucket (right). Both connected to a datalogger

Simultaneously with SWC monitoring, bulk precipitation was monitored hourly in an open patch in the forest by means of a tipping bucket (type RG1, Delta-T Devices) connected to a CR200 datalogger. However, to account for species-specific canopy interception loss (I_c), net precipitation (throughfall) was used as input in the Hydrus-1D model. This was calculated as P*(1 – I_c), with I_c being quantified *in situ* from April 2011 until April 2012 (see § 5.2.3). Species-specific transpiration was simulated using the MAESPA model (Duursma and Medlyn, 2012), based on net precipitation, global radiation, air temperature, relative humidity, wind speed, atmospheric pressure, groundwater levels, leaf area index (LAI), specific leaf area (SLA) and stem numbers. Global radiation, air temperature, relative humidity, wind speed and atmospheric pressure were measured by the Royal Meteorological Institute of Belgium at a weather station located 15 km from the study site. Species-specific LAI was measured via a direct method: after determining dry mass and specific leaf area (SLA) of 18 subsamples per species, the annual leaf litterfall (see § 4.2.1 and Table 4.4) was converted into LAI by multiplying by the SLA.

In the Hydrus-1D model, the soil was conceptualized to be homogeneous. The model was calibrated by means of the SWC values from the first monitoring year (March 2011 – February 2012), whereas the SWC values from the second monitoring year (March 2012 – February 2013)

were used to validate the model. Model calibration was done by optimizing the fit between modeled and observed SWC values, by varying K_{sat} and the soil water retention curve parameters θ_r θ_s and n, as these were the most sensitive parameters. Calibration and validation results for each tree species are shown in Appendix B. The model systematically overestimated the observed SWC values during the summer season, probably due to rather low simulated transpiration values (see § 7.4.1). Finally, the model was run with species-specific net precipitation and transpiration data of November 2009 – November 2010 to calculate soil water fluxes at 50 cm depth during the period when soil solution was sampled. As bulk precipitation was not measured *in situ* during that period, we used data of the Royal Meteorological Institute of Belgium from a measuring unit closeby. Groundwater levels under the forest were generally deeper than 2 m, so we modeled the soil water fluxes according to the assumption of free drainage. In one of the oak stands, we measured a consistently high groundwater level (average at 62 cm depth) and therefore this stand was omitted from further analyses.

7.2.4 Data analysis

Seepage fluxes were calculated by multiplying the modeled soil water fluxes at 50 cm depth by the measured soil solution concentrations per season. The sum of these seasonal seepage fluxes over the one year monitoring period (November 2009 – November 2010) yielded annual seepage fluxes. Since the lysimeters did not yield any soil solution samples during the summer period, annual seepage fluxes did not comprise summer seepage fluxes.

Base cation seepage fluxes were calculated by summing the equivalents of K^+, Ca^{2+}, Mg^{2+} and Na^+. Since Al^{3+} and NH_4^+ concentrations in the soil solution were negligible (majority of values lower than detection limits), they were not further considered in this study. Anion seepage fluxes were calculated by summing the equivalents of NO_3^-, SO_4^{2-}, PO_4^{3-} and Cl^-. Annual base cation and anion seepage fluxes, together with annual seepage fluxes of DOC, total and dissolved Cd and Zn and H^+ were considered as response variables.

The correlations between the annual seepage fluxes of Cd, Zn, base cations, anions, DOC and H^+, annual solution pH at 50 cm depth (average of seasonal values) and topsoil pH (0-5 cm, see Chapter 3) were calculated by means of Pearson correlation coefficients. Correlation analyses were performed in SPSS Statistics 22 for Windows.

The experimental design was hierarchical, with lysimeters grouped within stands and tree species replicated at the stand level, so we used multilevel models. The lmer function in the lme4 library of R version 3.1.1 was used here (Bates et al., 2011; R Core Team, 2014). First, we examined

whether there was a species effect on the seepage fluxes. Therefore, we constructed a null model with 'Stand' as random effect term. Next, we tested a model with the explanatory factor 'Species' as fixed effect against the null model with a likelihood ratio test to evaluate whether this factor significantly improved the null model. Finally, a full factorial model with 'Species' as fixed effect and 'Stand' as random effect term was fitted using restricted maximum likelihood estimates.

7.3 Results

Seasonal pH and concentrations of Cd, Zn, DOC, base cations and anions in the soil solution at 50 cm depth are reported in Appendix C.1. However, here we focus on the seepage fluxes (amounts), since fluxes are more important for the discussion of biogeochemical cycles. Seasonal and annual seepage fluxes are reported in Appendix C.2.

7.3.1 Soil water fluxes

The modeled soil water fluxes at 50 cm depth (Fig 7.1) were rather high, ranging from 312 mm to 757 mm or 78% to 92% of net precipitation (i.e. throughfall) during the modeled year. The interspecific differences seemed to be controlled mainly by the species-specific canopy interception losses, given the tight resemblance of the soil water fluxes with the net P values (Table 7.1).

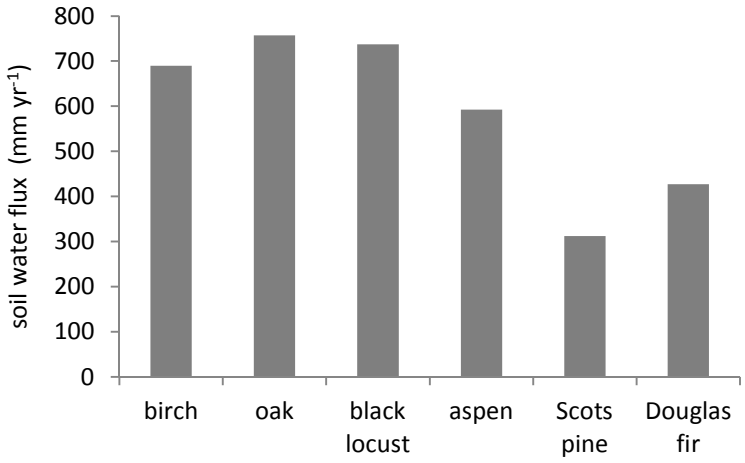


Figure 7.1 Modeled soil water fluxes at 50 cm depth under the six tree species (Nov 2009 – Nov 2010)

Table 7.1 shows input parameters used in the MAESPA model and in the Hydrus-1D model. Table 7.2 gives an overview of the main components of the evapotranspiration process, transpiration and canopy interception, and their relation to bulk precipitation. Canopy interception losses (I_c) were significantly higher for the coniferous species compared to the broadleaved species. I_c values ranged from 10.1 % (black locust) over 10.6 % (oak), 18.3 % (silver birch), 26.4 % (aspen), 43.6 % (Douglas fir) up to 56.5 % (Scots pine) (cfr. § 5.3.2). Simulated transpiration values were low and were related to LAI values, with highest values for Douglas fir and black locust.

Table 7.1 Species-specific input parameters for the MAESPA model (stem number, SLA, LAI, net precipitation P) and the model's output (simulated transpiration T)

	stem nr (trees ha^{-1})	SLA ($cm^2\ g^{-1}$)	LAI (-)	net P [§] (mm)	T [§] (mm)
silver birch	3565	163.1	2.2	753	92
oak	4525	127.8	4.0	824	111
black locust	3472	263.2	6.7	829	120
aspen	4167	119.1	2.8	679	101
Scots pine	5952	73.5	2.4	401	95
Douglas fir	3265	107.8	7.7	520	123

[§] During the modeled year (Nov 2009 – Nov 2010). Calculated net P and simulated T values were used to run the Hydrus-1D model for the studied year. See methods (§ 7.2.3) for details on net P calculations.

Table 7.2 Overview of simulated transpiration T and canopy interception I_c during the modeled year (Nov 2009 – Nov 2010) relative to bulk precipitation during that period (922 mm)

	T (mm)	T (% of bulk P)	I_c (mm)	$T + I_c$ (mm)	$T + I_c$ (% of bulk P)
silver birch	92	10.0	169	261	28.3
oak	111	12.0	98	209	22.6
black locust	120	13.0	93	213	23.1
aspen	101	11.0	243	344	37.4
Scots pine	95	10.3	521	616	66.8
Douglas fir	123	13.3	402	525	56.9

7.3.2 Element seepage fluxes

There was a significant species effect on annual seepage fluxes for all response variables (Fig 7.2).

Total and dissolved Cd and Zn fluxes in seepage water were highest under black locust and Scots pine. Douglas fir also revealed high total Cd fluxes, but for the dissolved Cd fluxes no significant differences could be found compared to the other species. Total Zn leaching under Douglas fir was slightly (yet not significantly) elevated, whereas dissolved Zn leaching under this conifer was low (Fig 7.2).

Highest base cation fluxes in seepage water were found under black locust. Base cation fluxes were also elevated under Scots pine and Douglas fir, being significantly higher than those under birch and aspen (only Scots pine). Ca^{2+} was the dominant cation in the seepage water for all considered tree species, constituting 60% – 75% of total base cation fluxes (Fig 7.2). A similar pattern as the base cation fluxes was found for the anion fluxes, being highest under black locust, lowest under the other three broadleaved species and intermediate under the two coniferous species. Particularly strong, positive correlations were indeed found between the base cation and anion seepage fluxes, and they were also strongly correlated with the Cd and Zn seepage fluxes (Table 7.3). The anion seepage flux under black locust was dominated by NO_3^-, whereas NO_3^- leaching contributed only a minor share under the other tree species. The five other species' anion seepage fluxes were mainly composed of SO_4^{2-} and Cl^-. Leaching of PO_4^{3-} was negligible under the considered tree species, except for oak (Fig 7.2).

Oak induced the highest DOC flux in the seepage water and silver birch showed an intermediate value. Surprisingly, DOC seepage fluxes were not correlated with any of the other elemental seepage fluxes (Table 7.3). H^+ leaching revealed a totally different pattern compared to DOC leaching; the highest H^+ flux was observed under Douglas fir and an intermediate value under black locust (Fig 7.2). H^+ fluxes were significantly (but less strong) correlated with Cd, base cation and anion fluxes, but not with the Zn fluxes (Table 7.3).

Fig 7.2 clearly shows that the considered tree species could be divided in two groups in terms of leaching losses. Black locust, Scots pine and Douglas fir were characterized by high seepage fluxes of Cd, Zn, base cations and anions, whereas these seepage fluxes were consistently low under silver birch, oak and aspen. DOC and H^+ seepage fluxes revealed a different pattern.

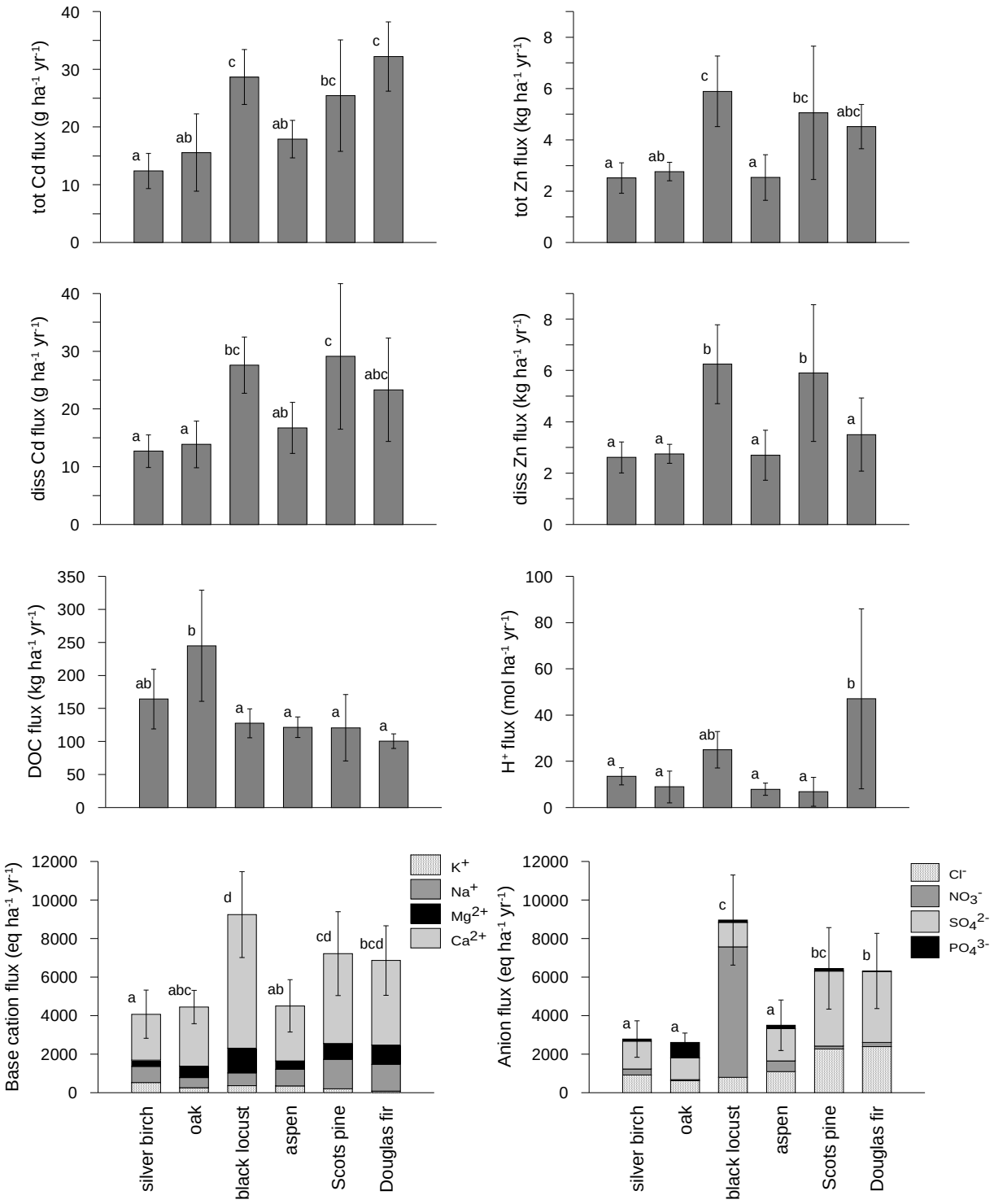


Figure 7.2 Annual seepage fluxes of total Cd and Zn, dissolved Cd and Zn, DOC, H+, base cations and anions at 50 cm depth under the six tree species (average ± st.dev); differences between species were tested using a multilevel model with a Bonferroni post hoc test, values with the same letter did not differ between species ($p < 0.05$)

Table 7.3 Pearson correlation coefficients of the correlation between annual element seepage fluxes and solution pH (pH_{sol}) at 50 cm depth and topsoil pH (n = 34)

	Cd_{tot}	Cd_{diss}	Zn_{tot}	Zn_{diss}	DOC	H^+	BC	Anions	pH_{sol}
Cd_{tot}									
Cd_{diss}	0.866 **								
Zn_{tot}	0.768 **	0.842 **							
Zn_{diss}	0.609 **	0.812 **	0.877 **						
DOC	- 0.222	- 0.155	- 0.261	- 0.142					
H^+	0.595 **	0.412 *	0.257	0.218	- 0.165				
BC	0.771 **	0.822 **	0.806 **	0.815 **	- 0.118	0.368 *			
Anions	0.794 **	0.832 **	0.845 **	0.820 **	- 0.259	0.388 *	0.980 **		
pH_{sol}	- 0.448 **	- 0.161	- 0.023	- 0.014	0.044	- 0.697 **	- 0.280	- 0.301	
$pH_{topsoil}$ §	- 0.213	- 0.290	- 0.409 *	- 0.266	- 0.137	- 0.207	- 0.388 *	- 0.390 *	0.237

§ Topsoil pH was determined in 2007 (cfr. § 3.2.2)

* $p < 0.05$; ** $p < 0.01$

7.4 Discussion

In order to get insight in the risk tree species exert on metal leaching to the groundwater, in metal polluted soils, we examined seepage fluxes under different tree species with diverging effects on biogeochemical and biophysical processes in the forest-soil system.

7.4.1 Soil water fluxes

The modeled soil water fluxes at 50 cm depth (Fig 7.1) were rather high, ranging from 312 mm to 757 mm or 78% to 92% of net P during the modeled year. The interspecific differences seemed to be controlled mainly by the species-specific canopy interception losses, given the tight resemblance of the soil water fluxes with the net P values (Table 7.1). No resemblance was found between the soil water fluxes and the basal area of the stands (see Table 3.1). Van der Salm et al. (2004) estimated the annual seepage fluxes under European forests to be on average 150 mm, with higher values in areas with high rainfall. The fairly low transpiration estimates (Table 7.1) might be one of the reasons for our high soil water fluxes. This hypothesis was supported by the fact that the model consistently overestimated the observed SWC values during the summer season, as observed during the calibration and validation process (see Appendix B).

The estimated transpiration values ranged from 10% to 13% of bulk P during the modeled year (Table 7.2). Other authors reported substantially higher values, with averages (both comprising coniferous and deciduous forests) around 33.5% in the UK (Nisbet, 2005) and 38% in Flanders (Verstraeten et al., 2005). Our low T values might potentially be due to limited water availability in the unsaturated zone of these dry sandy soils, and might suggest that the trees are also pumping up water from deeper soil layers or even groundwater. As the MAESPA model only accounted for soil water extraction from the unsaturated zone, the actual T values would probably be higher. However, given the aim of this study, i.e. estimating element leaching to deeper soil layers (> 50 cm) and the groundwater, only the flux in the unsaturated zone is of importance.

7.4.2 Element seepage fluxes

The element seepage fluxes were estimated by means of the modeled soil water fluxes, which were rather high. However, annual element seepage fluxes did not comprise summer seepage fluxes because the lysimeters did not yield any soil solution samples during the summer period. Hence, as the water balance model mainly overestimated the soil water fluxes during the summer

(see § 7.4.1 and Appendix B), we believe that the estimated seepage fluxes approach the reality quite reasonably. Nevertheless, modeling the soil water fluxes required some assumptions and relied, among others, on the simulated transpiration values, which in their turn also required some approximations. The absolute values of the element seepage fluxes should thus be interpreted with care. However, the relative differences between tree species are most relevant in the scope of this study, and we consider these to be reliable.

Patterns

The high similarity between the absolute values of total and dissolved Cd and Zn fluxes indicates that the metal fraction associated with inorganic and organic colloidal material in the soil solution was negligible.

Cd and Zn leaching at 50 cm depth was elevated under black locust, Scots pine and Douglas fir, compared to lower seepage fluxes under silver birch, oak and aspen. This pattern was significantly correlated with leaching of anions and base cations (Fig 7.2 and Table 7.3). The patterns indicate that Cd and Zn leaching was mainly related to the downward fluxes of anions and base cations (mainly Ca^{2+}), to a lesser extent to the H^+ fluxes, and not at all to the DOC seepage fluxes. It is generally known that transport of metals through the soil matrix is driven by a plethora of interrelated chemical reactions between the solid and the aqueous phase of the soil. Our results on Cd and Zn leaching corresponded broadly with patterns described in literature. Indeed, transport of metals through the soil matrix is generally enhanced by formation of soluble complexes with inorganic ligands, such as SO_4^{2-}, Cl^-, PO_4^{3-}, NO_3^-. Cd and Zn tend to complex mainly with Cl^- and SO_4^{2-} (Benjamin and Leckie, 1982; Bergkvist et al., 1989; McLean and Bledsoe, 1992; Ross, 1994). Also the presence of base cations in the soil solution may significantly increase the mobility of some metals, as there is competition between the major cations and free metal ions for adsorption onto binding sites (Cavallaro and McBride, 1978; McLean and Bledsoe, 1992; Sauvé et al., 2000; Voegelin et al., 2003). Especially the competition between Ca^{2+} and Cd^{2+} is distinct: increased Ca concentrations in the soil solution will displace Cd^{2+} ions from sorption sites and mobilize Cd (Christensen, 1984; Temminghoff et al., 1995). These processes were confirmed by our results.

In addition, a plethora of metal sorption studies have shown that solution pH has an overriding importance on metal solubility in many soils, by competing with H^+ ions for sorption sites (e.g. Bergkvist et al., 1989; Berthelsen et al., 1994; McBride et al., 1997; Sauvé et al., 2000a, 2000b; Strobel et al., 2001b, 2005; Voegelin et al., 2003; Degryse et al., 2007). Metal leaching can as well

be stimulated by formation of soluble complexes with organic ligands, such as DOC (Sauvé et al., 2000b; Strobel et al., 2001b, 2005). However, many authors found that soluble organic matter particularly has a strong affinity for Cu, Ni, Hg and Pb, whereas Cd and Zn tend not to complex strongly with soluble organics. This implies that the solubility of Cd and Zn is mainly controlled by pH with minor to no effects of DOC (e.g. Bergkvist et al., 1989; Holm et al., 1995; Römkens and de Vries, 1995; McBride et al., 1997; Sauvé et al., 2000b; Weng et al., 2001b; Strobel et al., 2001b, 2005). Our results on Cd and Zn leaching were consistent with these findings with respect to DOC. The effect of solution pH, on the other hand, was less pronounced in our study: solution pH was negatively correlated with total Cd seepage fluxes, yet its impact was less strong than that of the base cation and anion fluxes, and dissolved Cd fluxes and Zn fluxes were not affected by solution pH (Table 7.3). The relatively high solution pH at our study site (see Appendix C.1) might be the reason for this, as the critical solution pH for Cd and Zn breakthrough was not attained yet (see further).

Despite overall agreement in literature on the importance of pH on Cd and Zn leaching, we found the Cd and Zn seepage fluxes at our study site to be governed by leaching of anions and base cations (cfr. Fig 7.2 and Table 7.3). This might be caused by the fact that we are dealing with a young forest on post-agricultural soil. Due to the former liming and fertilization processes, the base cation status of the soil at our study site is still elevated (unreported data), but will decrease over time as base cation leaching proceeds. Solution pH is also still relatively high (see Appendix C.1), but we expect this to decrease with forest age, due to a build-up of the forest floor, especially for species with slowly decomposing litter (De Schrijver et al., 2012). It was shown that metal solubility increases rapidly when solution pH drops below a critical value. Cd and Zn leaching breakthrough was found to occur within the solution pH range of 4.0 to 4.5 (Bergkvist et al., 1989). This implies that the relative impact of base cation and anion leaching on Cd and Zn leaching at our study site might become weaker, whereas the effect of solution pH might become more important in the long term.

Topsoil pH was negatively correlated with total Zn seepage fluxes, but only quite weakly, and no correlations with dissolved Zn fluxes and Cd fluxes were found (Table 7.3). This shows that topsoil pH hardly affects metal leaching at 50 cm depth, and confirms the fact that metal transport through the soil matrix is dependent on multiple interrelated chemical reactions in the soil. Moreover, topsoil pH was not correlated with solution pH at 50 cm depth, implying that H^+ transport through the soil was also subjected to plenty of chemical reactions.

Finally, when comparing the pattern of the metal seepage fluxes (Fig 7.2) with that of the soil water fluxes (Fig 7.1), it was clear that Cd and Zn leaching losses at our study site showed no

resemblance with the downward soil water fluxes. This might imply that the differences in soil water fluxes between the tree species were not large enough to actually influence metal leaching, or else, that the impact of the chemical effects on Cd and Zn leaching (complexation with anions, competition for sorption sites with base cations and protons) was relatively more important than the effect of the biophysical processes (evapotranspiration, here represented by its main components $T + I_c$, see Table 7.2).

Tree species effects

Cd and Zn leaching was elevated under black locust, Scots pine and Douglas fir compared to lower seepage fluxes under silver birch, oak and aspen. This pattern was mainly driven by the seepage fluxes of anions and base cations (mainly Ca^{2+}).

The elevated anion flux under black locust was dominated by NO_3^-, which was not surprising since black locust is a N-fixing legume tree (Boring and Swank, 1984; Tateno et al., 2007; De Marco et al., 2013). It has been well documented that N input through symbiotic fixation of atmospheric N_2 results in increased NO_3^- leaching (Van Miegroet and Cole, 1984; Van Miegroet et al., 1992). The substantial amount of NO_3^- in black locust's seepage water also resulted in a high base cation flux under this tree species (Fig 7.2). The base cations are displaced from the exchange sites in the soil, and thus leached, as a consequence of the increase in ionic strength of the soil solution (Van Miegroet et al., 1992). The symbiotic N_2-fixation was most probably also responsible for the (slightly) elevated H^+ seepage flux under black locust. After all, N_2-fixation can enhance soil acidification as a by-product of increased nitrification rates (Van Miegroet and Cole, 1984). The resulting H^+ release may, in its turn, have contributed to black locust's high base cation seepage flux (Bowman et al., 2008).

The elevated anion fluxes under Scots pine and Douglas fir can be explained by the higher capacity of coniferous canopies to scavenge SO_4^{2-}, NO_3^- and Cl^- (dry deposition) compared to deciduous species (e.g. Bergkvist and Folkeson, 1995; Augusto et al., 2002, 2014; De Schrijver et al., 2007, 2008; Wuyts et al., 2008; Christiansen et al., 2010). The high efficiency of conifers in capturing atmospheric deposition can result in high losses of elements in seepage water (Bergkvist and Folkeson, 1995; Augusto et al., 2002, 2014; De Schrijver et al., 2007). De Schrijver et al. (2007) demonstrated, based on 38 case studies worldwide, comparing paired stands of pure coniferous and deciduous trees at sites with similar soil type and land use history, that the higher input flux of N and S clearly involved a higher seepage flux of NO_3^- and SO_4^{2-} under coniferous forests compared to deciduous forests. Moreover, the authors found a close relationship between

seepage of NO_3^- and SO_4^{2-} on the one hand, and K^+, Ca^{2+}, Mg^{2+} and Al^{3+} on the other hand. The higher seepage flux of anions under conifers thus clearly involved a higher soluble cation fraction, making total cation seepage a function of total anion seepage (Johnson, 1992).

Implications for phytostabilization

Phytostabilization uses plants to minimize the mobility and bioavailability of pollutants in the environment, either by stabilizing them or by preventing their migration (Smith and Bradshaw, 1972; Vangronsveld et al., 1995b). Consequently, tree species that induce Cd and Zn leaching to deeper soil layers and groundwater should be avoided.

Cd and Zn leaching at 50 cm depth showed to be mainly related to leaching of anions and base cations (mainly Ca^{2+}) and to a lesser extent to H^+ leaching/solution pH, at least at this young stage of forest development at a post-agricultural sandy site. We expect the effects of the different tree species on the seepage fluxes of anions, base cations and H^+ to evolve during the next decades. The relative impact of base cation and anion leaching on Cd and Zn leaching might become weaker, whereas the effect of solution pH might become more important in the long term. In this respect, we recommend not to plant tree species that generate high anion, base cation or H^+ fluxes when afforesting Cd and Zn contaminated lands, because these species pose a serious risk for metal leaching to deeper soil layers and potential contamination of the groundwater. In general, coniferous as well as N-fixing species should thus be avoided. At our study site, this was demonstrated by elevated Cd and Zn seepage fluxes at 50 cm under black locust, Scots pine and Douglas fir.

Contrary to what is often argued in literature about phytostabilization, we found that Cd and Zn leaching losses at our study site showed no resemblance with the downward soil water fluxes. This implies that evapotranspiration rates seemed to be a less decisive criterion for tree species selection on contaminated sites. Hence, when establishing a phytostabilization project on a contaminated sandy site, principal attention should be given to the aforementioned biogeochemical processes (complexation with anions, competition for sorption sites with base cations and protons).

Summarized, based on our results on Cd and Zn leaching, it seems that silver birch, oak and aspen may be planted on Cd and Zn contaminated sandy soils. However, as our previous findings showed a distinct Cd and Zn accumulation in aspen's foliage (see Chapter 3), this species is inappropriate for phytostabilization purposes because of the risk of above-ground metal dispersion.

7.5 Conclusions

Cd and Zn leaching at 50 cm depth was elevated under black locust, Scots pine and Douglas fir, compared to lower seepage fluxes under silver birch, oak and aspen. This pattern was significantly correlated with leaching of anions and base cations (mainly Ca^{2+}) and to a lesser extent to H^+ leaching, at least at this young stage of forest development at a post-agricultural sandy site. Transport of Cd and Zn through the soil matrix was enhanced by formation of soluble complexes with the anions on the one hand, and by competition for sorption sites with Ca^{2+} and H^+ on the other hand. DOC seepage fluxes were not correlated to the Cd and Zn seepage fluxes, because Cd and Zn do not tend to complex strongly with soluble organics. Due to former (agricultural) liming and fertilization processes, the base cation status of the soil at our study site is still elevated, but will decrease over time as base cation leaching proceeds. On the other hand, we expect H^+ leaching to increase with forest age. This implies that the relative impact of anion and base cation leaching on Cd and Zn leaching might become weaker, whereas the effect of solution pH might become more important in the long term.

Contrary to what is often argued in literature about phytostabilization, we found that Cd and Zn leaching losses at our study site showed no resemblance with the downward soil water fluxes. This might imply that the differences in soil water fluxes between the tree species were not large enough to actually influence metal leaching, or that the effect of evapotranspiration really was inferior to the biogeochemical effects in the soil (solution). These biogeochemical processes (complexation with anions, competition for sorption sites with Ca^{2+} and H^+) should thus get principal attention in phytostabilization projects on Cd and Zn contaminated sites.

We were able to solve one of the key questions involved in realizing a sustainable and ecologically sound phytostabilization project, namely 'which tree species will guarantee the lowest below-ground metal dispersion risks?'. It seems that silver birch, oak and aspen may be planted on Cd and Zn contaminated sandy soils. However, as our previous findings showed a distinct Cd and Zn accumulation in aspen's foliage, this species is inappropriate for phytostabilization purposes because of the risk of above-ground metal dispersion.

8

General discussion and conclusions

In the Campine region in north-eastern Belgium, an extended area of about 700 km² is diffusely polluted with mainly Cd and Zn, due to metal refining activities in the past. This historical metal pollution still causes human-toxicological and ecotoxicological risks through leaching to groundwater and dispersion in the food chain. Moreover, the Campine region is characterized by poor sandy soils, aggravating the risk for metal leaching and dispersion in the environment. Conventional soil sanitation techniques are technically and financially inadequate to tackle the pollution problem, because of its spatial extent and the relatively moderate contamination levels. Hence, alternative remediation strategies, such as phytoextraction and phytostabilization, should be investigated.

This thesis was executed in the context of the historical soil pollution in the Campine region. We evaluated the applicability and feasibility of both phytoextraction and phytostabilization of metal contaminated soils. **After profound evaluation of the opportunities, threats and perspectives of phytoextraction, we concluded that this technique is still far from practice at the present state of knowledge. However, even if the technique could be ameliorated, its applicability would be limited as metal uptake in plants poses serious risks for the environment and for contamination of the food chain.** Phytostabilization seems a more promising alternative for remediation of diffusely contaminated soils, as this technique aims at minimizing metal mobility and therefore decreases dispersion risks. However, phytostabilization cannot guarantee to completely exclude all dispersion risks. Hence, in this thesis, we focused on the phytostabilization technique and assessed its feasibility.

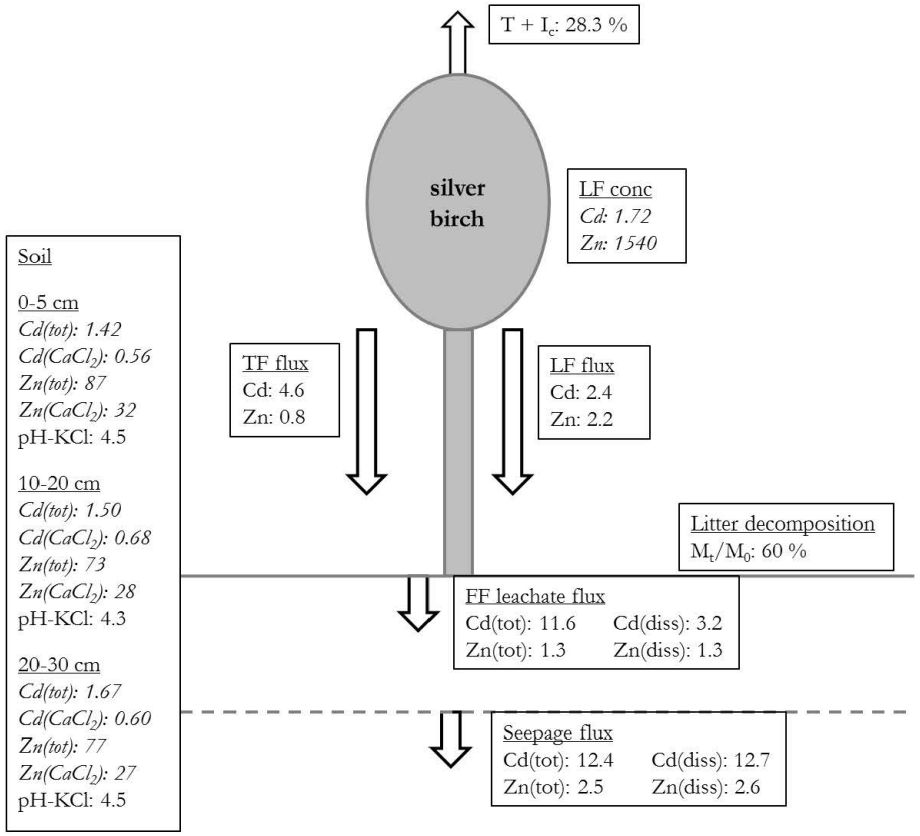
To accomplish sound phytostabilization projects, one should account for the associated risks of metal dispersion in the ecosystem. Sustainable phytostabilization requires the metals to be stabilized in the soil and should thus (aim to) avoid both above-ground (via uptake) and below-ground (via leaching) metal dispersion.
There is a clear tree species effect on metal uptake, on litter decomposition, on biogeochemical processes in the soil profile and on the water balance. Hence, as these processes are all inherent to metal cycling, the distribution and fluxes of metals in the forest-soil system will be tree species specific as well. Selecting appropriate tree species is thus crucial for achieving successful phytostabilization.

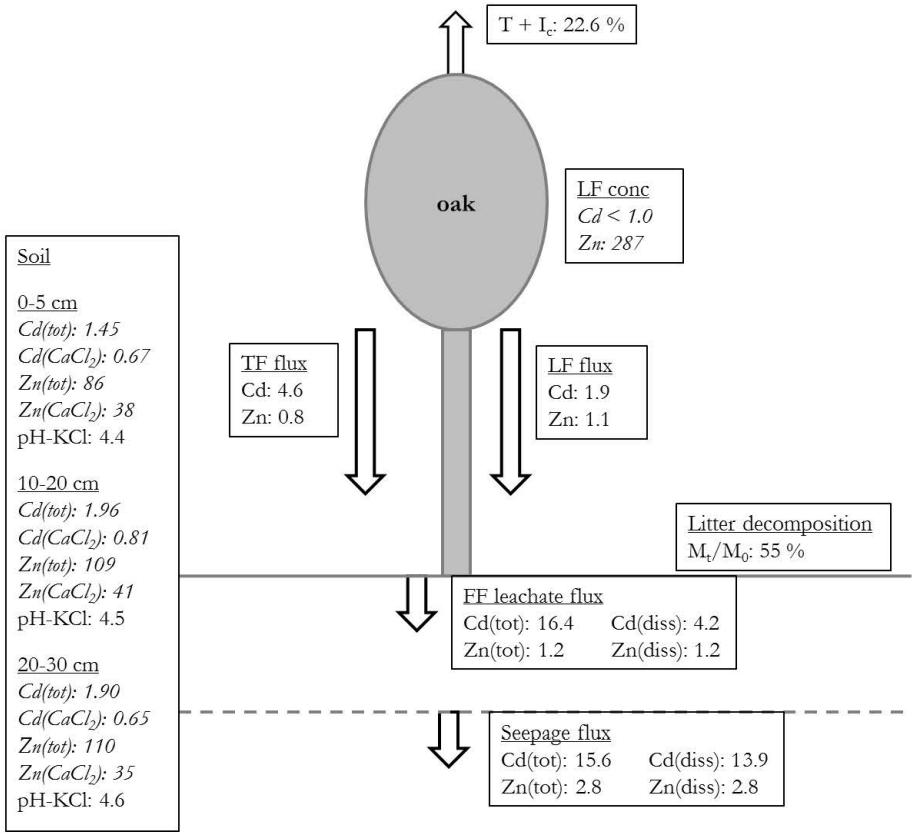
The main objectives of this thesis were to assess the feasibility of phytostabilization by afforesting diffusely Cd and Zn contaminated sandy soils and to determine the tree species effects on Cd and

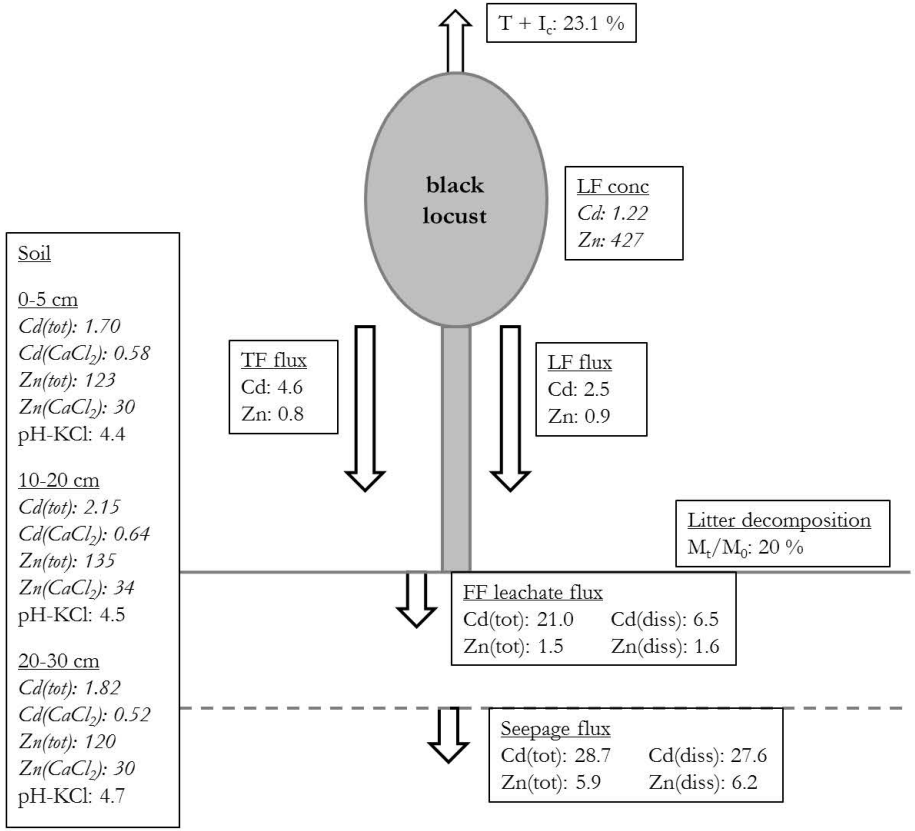
Zn cycling on these soils. Therefore, an observational study was carried out in a forest that was planted on a former agricultural, contaminated site in the Campine region (northeastern Belgium). The six considered tree species were silver birch (*Betula pendula*), oak (*Quercus robur* and *Q. petraea*), black locust (*Robinia pseudoacacia*), aspen (*Populus tremula*), Scots pine (*Pinus sylvestris*) and Douglas fir (*Pseudotsuga menziesii*). The trees were 10-15 years old during the sampling years between 2007-2012.

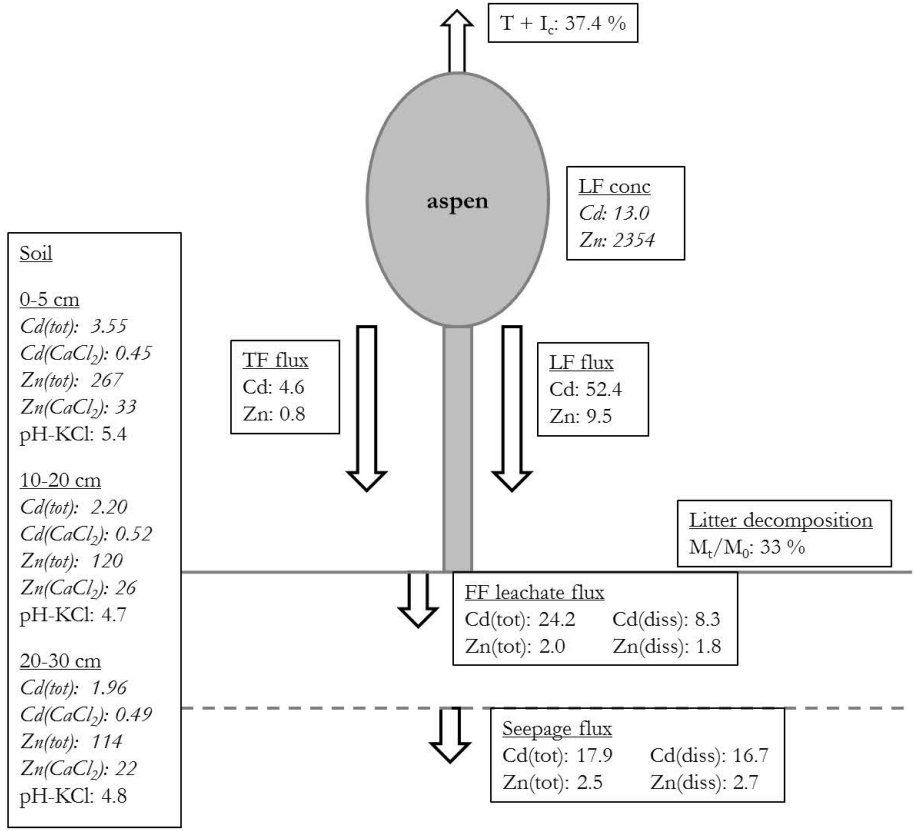
We were able to examine the main patterns involved in phytostabilization and to unravel the underlying processes. By understanding these processes and their interactions, we gained fundamental insights in the driving factors of metal cycling in forest ecosystems and in the influence of tree species choice on metal mobilization.

The main findings of this *in situ* study will be discussed below. Figure 8.1 compiles the tree species specific Cd and Zn fluxes and (re)distribution profiles in the forest ecosystem, together with litter decomposition dynamics and evapotranspiration ($T + I_c$), as measured at our study site during the course of this thesis.


T + I_c: 28.3 %
silver birch
LF conc
Cd: 1.72
Zn: 1540
TF flux
Cd: 4.6
Zn: 0.8
LF flux
Cd: 2.4
Zn: 2.2
Litter decomposition
M_t/M_0: 60 %
Soil
0-5 cm
Cd(tot): 1.42
Cd($CaCl_2$): 0.56
Zn(tot): 87
Zn($CaCl_2$): 32
pH-KCl: 4.5
10-20 cm
Cd(tot): 1.50
Cd($CaCl_2$): 0.68
Zn(tot): 73
Zn($CaCl_2$): 28
pH-KCl: 4.3
20-30 cm
Cd(tot): 1.67
Cd($CaCl_2$): 0.60
Zn(tot): 77
Zn($CaCl_2$): 27
pH-KCl: 4.5
FF leachate flux
Cd(tot): 11.6 Cd(diss): 3.2
Zn(tot): 1.3 Zn(diss): 1.3
Seepage flux
Cd(tot): 12.4 Cd(diss): 12.7
Zn(tot): 2.5 Zn(diss): 2.6


T + I_c: 22.6 %
oak
LF conc
Cd < 1.0
Zn: 287
TF flux
Cd: 4.6
Zn: 0.8
LF flux
Cd: 1.9
Zn: 1.1
Litter decomposition
M_t/M_0: 55 %
Soil
0-5 cm
Cd(tot): 1.45
Cd($CaCl_2$): 0.67
Zn(tot): 86
Zn($CaCl_2$): 38
pH-KCl: 4.4
10-20 cm
Cd(tot): 1.96
Cd($CaCl_2$): 0.81
Zn(tot): 109
Zn($CaCl_2$): 41
pH-KCl: 4.5
20-30 cm
Cd(tot): 1.90
Cd($CaCl_2$): 0.65
Zn(tot): 110
Zn($CaCl_2$): 35
pH-KCl: 4.6
FF leachate flux
Cd(tot): 16.4 Cd(diss): 4.2
Zn(tot): 1.2 Zn(diss): 1.2
Seepage flux
Cd(tot): 15.6 Cd(diss): 13.9
Zn(tot): 2.8 Zn(diss): 2.8


T + I_c: 23.1 %
black locust
LF conc
Cd: 1.22
Zn: 427
TF flux
Cd: 4.6
Zn: 0.8
LF flux
Cd: 2.5
Zn: 0.9
Litter decomposition
M_t/M_0: 20 %
FF leachate flux
Cd(tot): 21.0 Cd(diss): 6.5
Zn(tot): 1.5 Zn(diss): 1.6
Seepage flux
Cd(tot): 28.7 Cd(diss): 27.6
Zn(tot): 5.9 Zn(diss): 6.2
Soil
0-5 cm
Cd(tot): 1.70
Cd($CaCl_2$): 0.58
Zn(tot): 123
Zn($CaCl_2$): 30
pH-KCl: 4.4
10-20 cm
Cd(tot): 2.15
Cd($CaCl_2$): 0.64
Zn(tot): 135
Zn($CaCl_2$): 34
pH-KCl: 4.5
20-30 cm
Cd(tot): 1.82
Cd($CaCl_2$): 0.52
Zn(tot): 120
Zn($CaCl_2$): 30
pH-KCl: 4.7


T + I_c: 37.4 %
aspen
LF conc
Cd: 13.0
Zn: 2354
TF flux
Cd: 4.6
Zn: 0.8
LF flux
Cd: 52.4
Zn: 9.5
Litter decomposition
M_t/M_0: 33 %
FF leachate flux
Cd(tot): 24.2 Cd(diss): 8.3
Zn(tot): 2.0 Zn(diss): 1.8
Seepage flux
Cd(tot): 17.9 Cd(diss): 16.7
Zn(tot): 2.5 Zn(diss): 2.7
Soil
0-5 cm
Cd(tot): 3.55
Cd($CaCl_2$): 0.45
Zn(tot): 267
Zn($CaCl_2$): 33
pH-KCl: 5.4
10-20 cm
Cd(tot): 2.20
Cd($CaCl_2$): 0.52
Zn(tot): 120
Zn($CaCl_2$): 26
pH-KCl: 4.7
20-30 cm
Cd(tot): 1.96
Cd($CaCl_2$): 0.49
Zn(tot): 114
Zn($CaCl_2$): 22
pH-KCl: 4.8

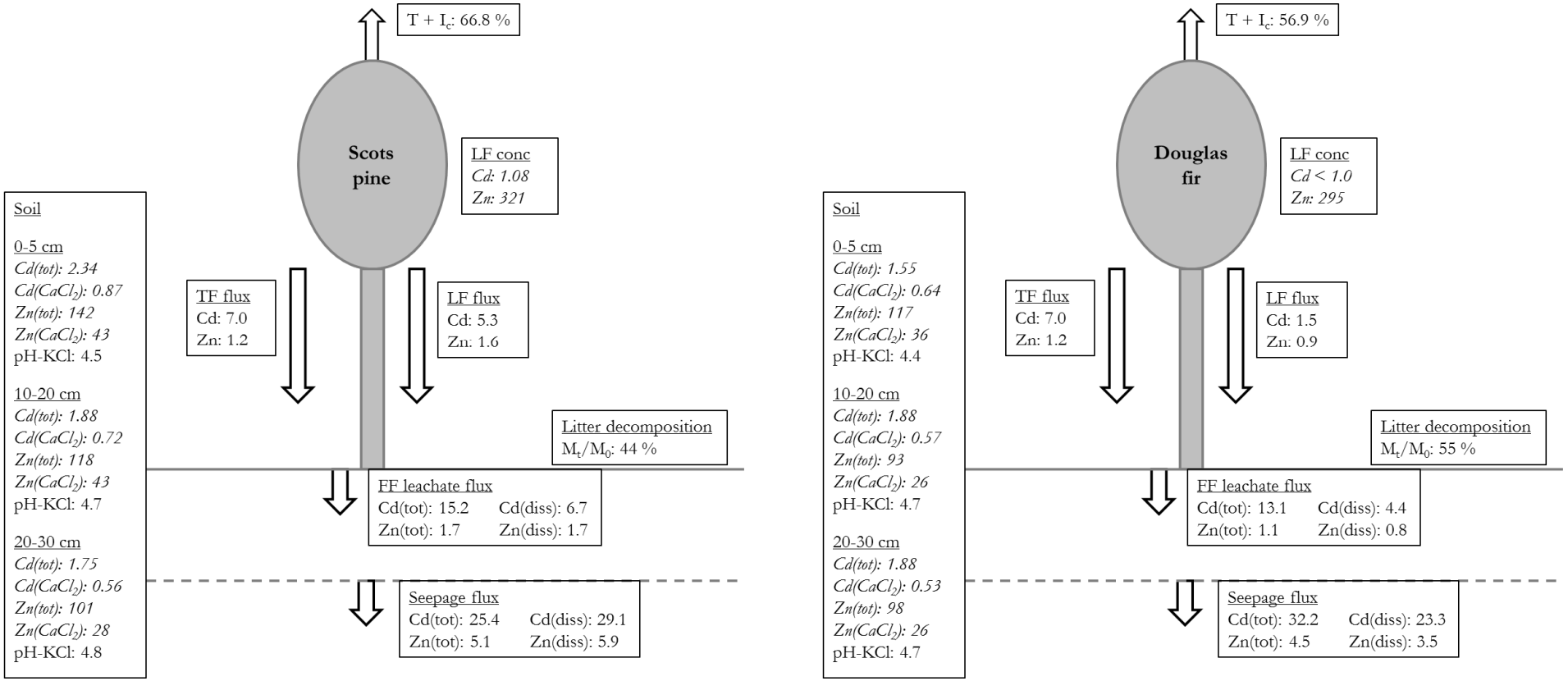


Figure 8.1 Overview of the Cd and Zn concentrations (mg kg^{-1} DM) (in italic) in the different soil depths and in the foliar litter (LF conc), soil pH-KCl (-), Cd (g ha^{-1} yr^{-1}) and Zn (kg ha^{-1} yr^{-1}) fluxes in foliar litterfall (LF flux), throughfall (TF flux[§]), forest floor leachate (FF leachate flux) and seepage water at 50 cm depth (seepage flux), transpiration + canopy interception ($T + I_c$ in % of bulk precipitation) and remaining mass in the litterbags after 20 months (M_t/M_0) as measured at our study site under the six tree species during the course of this thesis

[§] TF fluxes were not measured, but estimated based on average Cd and Zn concentrations in the air measured in the close vicinity of our study site (data of the Flemish Environment Agency), and deposition velocity (V_d) values of PM10 for coniferous and broadleaved species (literature values). TF fluxes were assumed to be equal to total depostion fluxes (assuming canopy exchange of Cd and Zn to be negligible).

8.1 Main findings

Dispersal of soil metals into the ecosystem can result from uptake of metals by the above-ground biomass, accumulation or mobilization of metals in the forest floor and topsoil, and leaching of metals towards groundwater.

8.1.1 Above-ground biomass

Aspen should be avoided when afforesting Cd and Zn contaminated lands, given its risks of above-ground metal dispersion via herbivory.

Aspen trees translocate high amounts of Cd and Zn into their foliage, generating a potential pathway of above-ground metal dispersion. This has long been demonstrated by numerous authors, for *Populus* spp. and *Salix* spp. in general (e.g. Brekken and Steinnes, 2004; Unterbrunner et al., 2007), and was confirmed by our results for aspen.

Metals accumulated in the leaves may pose a long-term risk to primary consumers and enter the food chain. According to Ross (1994), the input of metals into the food web is potentially more harmful than metal leaching into the groundwater.

Silver birch translocated considerable amounts of Zn to its leaves. However, as Zn is mainly phytotoxic, its human-toxicological and ecotoxicological risks are less radical compared to Cd. Based on our results, planting silver birch on Zn contaminated sites seems acceptable.

8.1.2 Forest floor and topsoil

The forest floor and topsoil are particularly vulnerable as they are the biologically most active parts of the soil system and biological activity has been shown to be highly sensitive to metal pollution (Bergkvist et al., 1989). A metal redistribution towards the forest floor and/or topsoil or mobilization of these metals is thus undesirable in phytostabilization projects.

Aspen induced an accumulation of total Cd and Zn concentrations in the topsoil (0-5 cm) and this after only 10 years of tree growth. However, the 'bioavailable' $CaCl_2$-extractable concentrations were low under aspen. The latter was caused by increased pH, CEC and OC content in the topsoil under the aspen trees.

Contaminated litterfall may result in a metal accumulation in the topsoil. Our results clearly demonstrate a significant increase of total Cd and Zn concentrations in the topsoil under aspen trees, compared to the deeper soil layers and the other tree species. This accumulation pattern is

expected to be continued in the future. Besides the total Cd and Zn accumulation, aspen was also the only tree species which significantly increased topsoil pH, CEC and OC content compared to the deeper soil layers and to the other tree species.

The differences in topsoil characteristics between the species were attributed to the chemical properties of the different leaf litter types. The elevated total Cd and Zn concentrations were obviously caused by the large Cd and Zn fluxes via aspen's foliar litterfall. Aspen also generated highest base cation litterfall fluxes and this was most probably the reason for the pH increase and for elevated organic matter contents in its topsoil and thus increased OC and CEC values. Although black locust was also characterized by high base cation concentrations in its leaf litter, no increase of topsoil pH under black locust was found. This was most likely due to the fact that 1) black locust is a N-fixing tree, and symbiotic N_2-fixation can lead to soil acidification as a by-product of increased nitrification rates and 2) its base cation litterfall fluxes were not elevated compared to most of the other tree species since black locust produced low leaf litterfall amounts.

As a consequence of increased pH, CEC and OC values, 'bioavailable' $CaCl_2$-extractable Cd and Zn concentrations appeared not to be elevated under aspen. Since extractable 'bioavailable' metal concentrations should preferably be considered for risk analysis, this may imply that -on the short term- the ecotoxicological risks linked with aspen might actually be smaller than expected from the accumulation of total Cd and Zn in the topsoil. However, NH_4OAc-EDTA-extractable Cd and Zn concentrations in the topsoil under aspen were elevated compared to other species and to deeper soil layers. This indicates that in more acid conditions the accumulated Cd and Zn in the topsoil will yet become more mobile and thus 'bioavailable'. This could for instance be the case when the aspen trees would be harvested and replaced by more acidifying species.

Silver birch translocated considerable amounts of Zn to its leaves, but this was not reflected in a Zn accumulation in the topsoil, probably due to its low leaf litterfall amounts. However, as litter biomass production will increase in time, Zn accumulation in the topsoil can potentially occur in the future.

During litter decomposition, 'high metal litter types' released part of their accumulated metals, whereas the 'low metal litter types' were characterized by a metal enrichment. Metal release from contaminated litter might involve risks for metal dispersion. On the other hand, metal enrichment of uncontaminated litter may be ecologically relevant as it can be easily transported or serve as food source.

Since aspen and silver birch are the only species that showed elevated Cd and/or Zn concentrations in their leaf litter, we considered those two species as 'high metal litter types'. The other (non-accumulating) *in situ* species and *ex situ* uncontaminated aspen and birch were considered as 'low metal litter types'.

A litterbag experiment lasting for 30 months revealed that the 'low metal litter types' were characterized by an increase in Cd and Zn amounts during the decomposition process. Such an increase in absolute amounts requires a net transport of Cd and Zn into the litterbags from external sources, such as atmospheric deposition and throughfall, microbial translocation and immobilization of metals from the underlying contaminated soil (mainly by fungi), or direct contact of contaminated soil particles with the litter. We assume that the observed metal enrichment was induced by a combination of aforementioned processes. The fact that the 'low metal litter types' became metal-enriched when they decomposed on a contaminated site, implies that the organic matter of decomposing leaves and needles acted as an efficient metal storage pool. Leaf litter can thus act as a temporary sink for metals from the soil around and below the litter. Such upward metal enrichment may be ecologically relevant as litter can be transported easily to other sites (e.g. by wind) or serve as a source of food for a variety of organisms.

On the other hand, our litterbag experiment revealed that aspen litter released 50% of its initial Cd amount and 41% of its initial Zn amount during the course of the experiment. There was also a slight decrease (9%) of the Zn amount in birch litter, yet this was not significant compared to the Zn dynamics in the other species. The external sources of metal input into decomposing litter, as described above, must have been negligible compared to the elevated initial metal amounts in the aspen and birch litter. Hence, metal output was faster than the input.

Base cations, N and C were released from all litter types during the decomposition process. The metal and nutrient releases from decomposing litter were mainly reflected in topsoil characteristics (Cd and Zn concentrations, CEC, OC content), but they were hardly related to forest floor leachate fluxes (Cd, Zn, DOC, H^+).

Despite substantial Cd and Zn accumulation in aspen's leaf litter and significant Cd and Zn release during its litter decomposition, Cd and Zn leaching from the forest floor under aspen was not as high as foreseen. Forest floor leachate fluxes were hardly related to metal and nutrient dynamics in decomposing litter.

Forest floor (FF) leachates were sampled with zero-tension lysimeters. We found that total Cd fluxes in forest floor leachate under aspen were slightly higher than those in the other species' leachates, yet the relative differences between the species were considerably smaller when looking at dissolved Cd fluxes. Moreover, no tree species effect was found for Zn leachate fluxes. We had expected higher metal FF leachate fluxes under aspen, given its elevated Cd and Zn input via leaf litterfall (respectively 10 - 34 and 4 - 11 times higher compared to the other tree species) as well as significant Cd and Zn release during its litter decomposition. As discussed before, the high Cd and Zn LF fluxes under aspen gave rise to a distinct Cd and Zn accumulation in its topsoil, but this was not univocally reflected in higher Cd and Zn FF leachate fluxes. We propose that, given the former agricultural practices on the site, this discrepancy might be caused by high activity of soil biota, for example burrowing earthworms, mixing the topsoil with contaminated litter. In any case, the fact that metal leaching from the forest floor under aspen was not as high as foreseen, might imply that the risks of below-ground metal dispersion under aspen might actually be smaller than expected. This will be discussed more in detail in § 8.1.3.

For the other tree species, on the contrary, we found Cd fluxes in the forest floor leachates to be considerably higher than Cd fluxes in litterfall. Rough estimates of throughfall (see Fig 8.1 for details on estimation procedure) revealed that the throughfall Cd fluxes might be partially responsible for this discrepancy. This insight emphasizes the relevance of throughfall measurements within future research.

Furthermore, our results showed a significant tree species effect on H^+, DOC and base cation fluxes, with especially very low H^+ fluxes in aspen leachate (130 times less than oak). It seems that the extremely low proton amounts leaching from aspen's forest floor resulted in low Cd and Zn mobilization in the topsoil under aspen trees: despite significant accumulation of total Cd and Zn concentrations in its topsoil, we found that its immediately bioavailable ($CaCl_2$-extractable) Cd and Zn topsoil concentrations were not elevated. Scots pine and oak were characterized by high H^+ and DOC fluxes as well as low base cation fluxes in their forest floor leachates, suggesting that those species might enhance (top)soil acidification, and thus bear a potential risk for below-ground metal dispersion. However, significant changes in total or immediately bioavailable ($CaCl_2$-extractable) Cd and Zn concentrations in their topsoil, compared to deeper soil layers, were not (yet) found in this young forest.

It is often postulated that leaf litter quality has a direct link with the chemical composition of the forest floor leachate (Strobel et al., 2001a). However, our results reveal that this link was not so distinct. Firstly, the H^+ and DOC forest floor leachate fluxes were not correlated with leaf litter quality (here expressed by base cation LF fluxes and C/N ratio). Secondly, Cd and Zn forest floor leachate fluxes were correlated with the LF fluxes of Cd and Zn, respectively, but we found a large discrepancy between the metal LF fluxes and the FF leachate fluxes, for all species. Summarized, at our study site, leaf litter quality did have a clear effect on topsoil characteristics (Cd and Zn concentrations, pH, CEC and OC; see higher) but its effect on the the forest floor leachate fluxes was not so unambiguous.

Planting 'nutrient-rich' shrub species in pine and oak stands on poor and acidic sandy soils will not change topsoil conditions related to soil acidification. Adequate tree species selection (or conversion of pine towards deciduous tree species) seems more effective to enhance topsoil properties as pH and base saturation than planting shrubs under tree species with nutrient-poor leaf litter.

We know from previous research and from our own observations that 1) soil pH has an univocal effect on Cd and Zn solubility, and hence mobilization (e.g. Berthelsen et al., 1994; McBride et al., 1997; Sauvé et al., 2000a, 2000b; Degryse et al., 2007) and that 2) litter quality affects topsoil characteristics (e.g. De Schrijver et al., 2012). Typical tree species on the nutrient-poor sandy soils in the Campine region (e.g. pine, oak) are often characterized by nutrient-poor leaf litter, enhancing soil acidification and thus metal mobilization. It is often postulated that topsoil acidification under 'acidifying' tree species can be counteracted by admixing shrub species with nutrient-rich leaf litter. Therefore, in the light of sustainable metal stabilization, we investigated whether topsoil conditions related to soil acidification (pH, base saturation, CEC) in pine and oak stands can be altered by the presence of a 'nutrient-rich' shrub layer. The shrub species included were European rowan (*Sorbus aucuparia*), alder buckthorn (*Rhamnus frangula*) and black cherry (*Prunus serotina*). Moreover, we aimed to determine the threshold cover of the shrubs needed to obtain a significant effect in the topsoil.

Despite the fact that the shrubs produced litter with significantly higher base cation and N concentrations than that of the studied tree species, we did not find any significant changes in topsoil conditions in the pine and oak stands under study, even under dense shrub layers (87 – 91 % cover). Consequently, it seems thus not feasible to mitigate soil acidification by introducing a shrub layer with nutrient-rich leaf litter in pine and oak stands on poor sandy soils. Our results were in line with previous findings of Hommel et al. (2007), who stated that litter quality was the

key factor to restore the buffering capacity of forest topsoils, yet this mechanism was only successful within a range of 'intermediate' (moderately poor) sites. Sandy soils should e.g. contain at least 15% silt to be susceptible for the 'nutrient-rich litter effect'.

On the other hand, we found a tree species (stand type) effect, with 'nutrient-richer' litterfall in the oak stands compared to the pine stands. This was reflected in less humus build-up (thinner FH-horizon) and higher pH, CEC_e, BS and lower C/N in the topsoil in the oak stands compared to the pine stands. Adequate tree species selection (or conversion of pine towards deciduous tree species) seems thus more effective to mitigate soil acidification than planting shrubs under tree species with nutrient-poor leaf litter.

8.1.3 Seepage water

Cd and Zn leaching at 50 cm depth was elevated under black locust, Scots pine and Douglas fir, compared to lower seepage fluxes under silver birch, oak and aspen. This pattern was significantly correlated with leaching of anions and base cations, but not linked with evapotranspiration.

We were able to solve one of the key questions involved in realizing a sustainable and ecologically sound phytostabilization project, namely 'which tree species will guarantee the lowest below-ground metal dispersion risks?' Moreover, we unraveled the driving processes behind the Cd and Zn seepage fluxes under the six tree species at our study site.

Cd and Zn leaching showed to be mainly related to leaching of anions and base cations (mainly Ca^{2+}) and to a lesser extent to H^+ leaching, at least at this young stage of forest development at a post-agricultural sandy site. Transport of Cd and Zn through the soil matrix was thus enhanced by formation of soluble complexes with the anions on the one hand, and by competition for sorption sites with Ca^{2+} and H^+ on the other hand. DOC seepage fluxes were not correlated to the Cd and Zn seepage fluxes, because Cd and Zn do not tend to complex strongly with soluble organics. Due to former (agricultural) liming and fertilization processes, the base cation status of the soil at our study site is still elevated, but will decrease over time as base cation leaching proceeds. On the other hand, we expect H^+ leaching to increase with forest age, due to a build-up of the forest floor, especially for species with slowly decomposing litter. This implies that the relative impact of base cation and anion leaching on Cd and Zn leaching might become weaker, whereas the effect of pH might become more important in the long term.

Contrary to what is often argued in literature about phytostabilization, we found that Cd and Zn leaching losses at our study site showed no resemblance with the downward soil water fluxes.

This might imply that the differences in soil water fluxes between the tree species were not large enough to actually influence metal leaching, or else, that the impact of the biogeochemical processes in the soil (solution) on Cd and Zn leaching (complexation with anions, competition for sorption sites with base cations and protons) was relatively more important than the effect of evapotranspiration.

Table 8.1 compiles the different biogeochemical and biophysical processes inherent to metal leaching, clearly showing the aforementioned patterns: an overriding importance of base cation and anion leaching, an overall agreement with H^+ leaching, but no resemblance with DOC leaching nor with evapotranspiration (here represented by its main components $T + I_c$).

Table 8.1 Schematic overview of the processes affecting metal leaching, showing relative values (relative to maximum value) for each process, with the tree species divided in a group of high (red) and low (green) Cd and Zn leachers

	H^+ leaching	DOC leaching	BC & Anion leaching	$T + I_c$
silver birch	0.3	0.7	0.4	0.4
oak	0.2	1.0	0.4	0.3
aspen	0.2	0.5	0.4	0.6
black locust	0.5	0.5	1.0	0.3
Scots pine	0.1	0.5	0.8	1.0
Douglas fir	1.0	0.4	0.7	0.9

Finally, we would like to add that the relatively low Cd and Zn seepage fluxes under aspen confirm that the risks of below-ground Cd and Zn dispersion under this tree species are indeed smaller than expected from the accumulation of total Cd and Zn in its topsoil. The low immediately bioavailable ($CaCl_2$-extractable) Cd and Zn concentrations in its topsoil and the lower than foreseen Cd and Zn fluxes in its forest floor leachate suggested this already (see above), but our results on metal leaching at 50 cm depth could thus confirm our presumptions.

8.2 Implications for forest management

Sustainable phytostabilization projects require the metals to be stabilized in the soil and should thus aim at minimizing metal dispersion via both above-ground and below-ground pathways. The main findings of this thesis (§ 8.1) provide a scientific basis of high relevance for management of metal contaminated sandy soils. Translating our main findings to field practices results in following recommendations:

Tree species that accumulate metals in their foliar biomass should be avoided.

Metals accumulated in the leaves may pose a long-term risk to primary consumers and enter the food web. Besides, contaminated litterfall may give rise to an accumulation of metals in the topsoil. This could provide a new exposure pathway for the metals, especially since the topsoil is highly vulnerable because the majority of biological life is concentrated there. In addition, the accumulated metals in the topsoil may be mobilized under acidifying conditions (e.g. when trees are harvested and replaced by acidifying species), potentially resulting in leaching towards deeper soil layers.

On Cd and Zn contaminated sites, *Populus* species and *Salix* species should thus be avoided (e.g. Mertens et al., 2001; Brekken and Steinnes, 2004; Unterbrunner et al., 2007; Van Slycken, 2011). Our results confirmed this, as we demonstrated a significant Cd and Zn accumulation in aspen foliage.

Silver birch translocated considerable amounts of Zn to its leaves. However, as Zn is mainly phytotoxic, its human-toxicological and ecotoxicological risks are less radical compared to Cd. Based on our results, planting silver birch on Zn contaminated sites seems acceptable.

Tree species that induce substantial leaching of anions should be avoided.

We showed that anion leaching induced leaching of base cations, and that seepage of both anions and base cations (mainly Ca^{2+}) resulted in elevated Cd and Zn seepage fluxes at 50 cm depth. Tree species that are characterized by high anion and base cation losses, pose thus a serious risk for metal leaching to deeper soil layers and potential contamination of the groundwater.

In general, coniferous as well as N-fixing species should thus be avoided. At our study site, this was demonstrated by elevated Cd and Zn seepage fluxes at 50 cm under black locust, Scots pine and Douglas fir.

Tree species that are characterized by low soil solution pH should be avoided.

The soil solution pH has a major effect on Cd and Zn solubility, due to competition between metal and H^+ ions for sorption sites. This implies that species with low soil solution pH also induce higher Cd and Zn leaching, and thus pose a considerable risk for metal contamination of the groundwater.

In our study, the impact of soil solution pH on Cd and Zn leaching was less pronounced, which was most probably attributed to the young stage of forest development on a post-agricultural site (with high initial pH and Ca contents). The solution pH at 50 cm depth at our study site (see Appendix C.1) was still higher than the critical value for Cd and Zn breakthrough, which was found to occur within a solution pH range of 4.0 to 4.5 (Bergkvist et al., 1989). However, we expect the solution pH to decrease with forest age, especially for species with slowly decomposing litter, implying that the effect of pH on Cd and Zn leaching will become more important in the long term.

In general, tree species characterized by slow litter decomposition rates (and thus substantial humus build-up) as well as N-fixing species should be avoided, as these are acidifying processes. At our study site, this was confirmed by elevated Cd and Zn seepage fluxes under black locust, Scots pine and Douglas fir.

Evapotranspiration rates seem to be a less decisive criterion for tree species selection on contaminated sites.

Comparison of the Cd and Zn seepage fluxes with the soil water fluxes under the different tree species at our study site revealed that Cd and Zn leaching losses showed no resemblance with the downward soil water fluxes. The impact of the biogeochemical processes in the soil (solution), i.e. complexation with anions, competition for sorption sites with base cations (mainly Ca^{2+}) and H^+ ions, was relatively more important than the effect of evapotranspiration. Hence, when establishing a phytostabilization project on a contaminated sandy site, principal attention should be given to the aforementioned biogeochemical processes.

Planting 'nutrient-rich' shrub species under 'acidifying' tree species on poor and acidic sandy soils is not effective to counteract topsoil acidification.

Our results revealed that dense shrub layers (87 – 91 % cover) of European rowan, alder buckthorn and black cherry in pine and oak stands, on poor and acidic sandy soils, did not affect topsoil conditions related to soil acidification. Adequate tree species selection (or conversion of

pine towards deciduous tree species) seems more effective to mitigate soil acidification than planting shrubs under tree species with nutrient-poor leaf litter.

Summarized, taking into account all the aforementioned recommendations, it seems that silver birch and oak may be planted on Cd and Zn contaminated sandy soils. Aspen, black locust, Scots pine and Douglas fir, on the other hand, cause risks for above-ground or below-ground metal dispersion and should therefore be avoided on Cd and Zn contaminated sandy sites.
These conclusions are drawn from our study in a young forest (10 – 15 years). Therefore, we would like to add that it is unsure whether they can be extended to the long term. Further research in older forests is thus essential (see below).

8.3 Recommendations for further research

The effects of the different tree species on the (top)soil characteristics, on the metal redistribution in the soil profile and on the forest floor leachate and seepage fluxes will evolve during the next decades and will probably become more pronounced in the future. Therefore, in order to get an idea of tree species effects on metal cycling in the ecosystem on the long term, it is essential to execute similar research in older forests on contaminated sites. Unfortunately, long-term afforestations of contaminated sites are scarce. However, chronosequences with different tree species on uncontaminated sites may reveal relevant insights in the processes affecting metal mobilization, e.g. (top)soil acidification or seepage fluxes of base cations, anions, H^+, DOC.

Our study was carried out in a post-agricultural forest. Due to the former liming and fertilization practices, the site was still relatively well-buffered, compared to other sandy forest sites without agricultural background. This was reflected in an elevated base cation status in the soil (mainly Ca) and in a relatively high solution pH (higher than the critical value for Cd and Zn breakthrough). As the soil (solution) status has an unambiguous effect on metal behaviour, it would be very interesting to investigate metal cycling at less buffered sandy sites. This would also give an idea of how the metal behaviour will potentially evolve over time (as base cation leaching proceeds and solution pH drops below the critical value for Cd and Zn breakthrough) in post-agricultural forests.

Our results on Cd and Zn leaching losses revealed that they showed no resemblance with the downward soil water fluxes. This might imply that the differences in soil water fluxes between

the tree species were not large enough to actually influence metal leaching, or that the effect of evapotranspiration really was inferior to the biogeochemical effects in the soil (solution). Therefore we suggest to examine differences in metal seepage fluxes between forest and semi-natural open landscapes (grassland, heathland). Soil water fluxes under open landscapes are considerably larger than under forests, so higher metal seepage fluxes under non-forested sites are to be expected. However, if it would be confirmed in open landscapes that metal leaching is indeed governed by the biogeochemical processes in the soil, this could potentially reveal lower metal seepage fluxes under grassland or heathland than under forest, as grasslands and heathlands are characterized by less leaching of anions, base cations, H^+ compared to forests.
In addition, as modeled soil water fluxes are always rough approximations of the reality, a more accurate quantification of evapotranspiration rates would be opportune. This could for instance be achieved by means of sap flow measurements or eddy covariance techniques.

This thesis revealed fundamental insights into terrestrial Cd and Zn cycling, but we did not consider the effective impact on the environment and on humans. More research needs to be done regarding the actual effects of metal fluxes in the forest-soil system on public health and on ecological food webs.

Appendix

Appendix A

Table A.1 Seasonal leachate pH and concentrations of Cd and Zn (total and dissolved), DOC and base cations (BC) in forest floor leachates under the six tree species (n = 6) (average ± st.dev)

	season §	pH (-)	Cd_{tot} ($\mu g\ l^{-1}$)	Cd_{diss} ($\mu g\ l^{-1}$)	Zn_{tot} ($mg\ l^{-1}$)	Zn_{diss} ($mg\ l^{-1}$)	DOC ($mg\ l^{-1}$)	BC ($meq\ l^{-1}$)
silver birch	autumn	6.85 ± 0.35	1.0 ± 0.3	0.3 ± 0.1	0.14 ± 0.05	0.14 ± 0.05	11.3 ± 2.9	0.58 ± 0.10
	winter	6.68 ± 0.19	1.4 ± 1.4	0.2 ± 0.1	0.11 ± 0.04	0.10 ± 0.05	4.3 ± 0.6	0.35 ± 0.06
	spring	6.61 ± 0.18	2.8 ± 0.7	0.6 ± 0.2	0.29 ± 0.05	0.30 ± 0.06	15.7 ± 2.7	0.64 ± 0.21
	summer	6.02 ± 1.10	2.0 ± 0.6	0.7 ± 0.3	0.25 ± 0.16	0.26 ± 0.06	13.1 ± 1.6	0.58 ± 0.09
oak	autumn	6.40 ± 0.20	1.1 ± 0.7	0.5 ± 0.3	0.16 ± 0.06	0.16 ± 0.06	20.5 ± 6.7	1.12 ± 0.22
	winter	6.46 ± 0.33	3.1 ± 1.2	0.2 ± 0.2	0.09 ± 0.04	0.08 ± 0.03	8.3 ± 2.0	0.41 ± 0.21
	spring	5.91 ± 0.89	2.3 ± 1.3	0.7 ± 0.4	0.18 ± 0.07	0.19 ± 0.07	20.4 ± 6.3	0.67 ± 0.27
	summer	4.73 ± 0.45	1.8 ± 1.2	0.8 ± 0.6	0.21 ± 0.11	0.22 ± 0.12	18.0 ± 2.6	0.77 ± 0.59
black locust	autumn	6.70 ± 1.12	1.3 ± 0.5	1.0 ± 0.6	0.21 ± 0.09	0.21 ± 0.10	16.7 ± 2.3	1.47 ± 0.33
	winter	6.61 ± 0.37	3.2 ± 1.3	0.4 ± 0.1	0.10 ± 0.02	0.10 ± 0.02	8.4 ± 3.1	0.71 ± 0.17
	spring	6.04 ± 0.63	5.3 ± 1.7	1.3 ± 1.2	0.33 ± 0.29	0.35 ± 0.31	19.4 ± 7.6	1.28 ± 0.94
	summer	5.58 ± 1.02	2.5 ± 0.9	1.0 ± 0.7	0.25 ± 0.14	0.25 ± 0.15	12.5 ± 4.8	1.10 ± 0.51

Table A.1 (continued)

aspen	autumn	7.54 ± 0.28	2.1 ± 1.5	1.2 ± 0.8	0.27 ± 0.21	0.18 ± 0.07	14.1 ± 2.5	1.68 ± 0.62
	winter	7.24 ± 0.21	4.0 ± 1.1	1.0 ± 0.6	0.19 ± 0.10	0.19 ± 0.11	7.2 ± 2.5	1.12 ± 0.46
	spring	6.92 ± 0.51	5.3 ± 0.9	1.7 ± 0.9	0.51 ± 0.33	0.52 ± 0.34	21.1 ± 5.2	1.42 ± 0.35
	summer	6.92 ± 0.12	4.3 ± 1.3	1.6 ± 0.7	0.41 ± 0.19	0.38 ± 0.14	15.1 ± 2.3	1.20 ± 0.19
Scots pine	autumn	6.23 ± 0.33	2.4 ± 1.1	2.3 ± 1.1	0.44 ± 0.20	0.48 ± 0.22	33.8 ± 9.0	0.89 ± 0.21
	winter	6.01 ± 0.48	4.8 ± 0.5	1.2 ± 0.6	0.24 ± 0.09	0.25 ± 0.10	15.4 ± 6.3	0.58 ± 0.25
	spring	6.49 ± 1.23	5.3 ± 1.0	2.4 ± 1.2	0.47 ± 0.19	0.52 ± 0.24	31.3 ± 6.6	1.30 ± 0.47
	summer	4.61 ± 0.23	4.1 ± 1.0	2.5 ± 0.8	0.60 ± 0.17	0.60 ± 0.16	33.5 ± 7.3	1.21 ± 0.28
Douglas fir	autumn	5.90 ± 1.01	2.0 ± 2.0	0.6 ± 0.4	0.42 ± 0.48	0.13 ± 0.09	18.5 ± 4.3	1.14 ± 0.91
	winter	6.73 ± 0.45	3.9 ± 0.6	1.3 ± 1.5	0.11 ± 0.05	0.11 ± 0.06	8.6 ± 3.0	0.75 ± 0.30
	spring	6.10 ± 0.80	3.6 ± 1.5	0.9 ± 0.7	0.20 ± 0.12	0.22 ± 0.16	21.0 ± 4.3	1.26 ± 0.55
	summer	5.71 ± 0.62	1.8 ± 1.1	0.8 ± 0.5	0.21 ± 0.08	0.20 ± 0.09	25.6 ± 7.0	1.37 ± 0.66

§ autumn: 19/09/2009 - 30/11/2009; winter: 01/12/2009 - 31/03/2010; spring: 01/04/2010 - 24/06/2010; summer: 25/06/2010 - 21/09/2010 (see § 5.2.2)

Table A.2 Seasonal and annual fluxes of Cd and Zn (total and dissolved), DOC, H^+ and base cations (BC) in forest floor leachates under the six tree species (n = 6) (average ± st.dev)

	season §	Cd_{tot} (g ha^{-1} yr^{-1})	Cd_{diss} (g ha^{-1} yr^{-1})	Zn_{tot} (kg ha^{-1} yr^{-1})	Zn_{diss} (kg ha^{-1} yr^{-1})	DOC (kg ha^{-1} yr^{-1})	H^+ (mol ha^{-1} yr^{-1})	BC (eq ha^{-1} yr^{-1})
silver birch	autumn	1.8 ± 0.5	0.6 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	19.8 ± 5.1	0.34 ± 0.36	1021 ± 175
	winter	3.1 ± 3.2	0.5 ± 0.3	0.2 ± 0.1	0.2 ± 0.1	9.7 ± 1.3	0.50 ± 0.23	785 ± 135
	spring	2.0 ± 0.5	0.4 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	10.9 ± 1.9	0.18 ± 0.07	444 ± 144
	summer	4.8 ± 1.5	1.6 ± 0.6	0.6 ± 0.1	0.6 ± 0.1	31.8 ± 3.9	14.1 ± 21.4	1404 ± 215
	annual	11.6 ± 3.1	3.2 ± 0.9	1.3 ± 0.3	1.3 ± 0.3	72.2 ± 7.5	15.2 ± 21.3	3653 ± 430
oak	autumn	2.2 ± 1.3	1.0 ± 0.5	0.3 ± 0.1	0.3 ± 0.1	39.6 ± 12.9	0.83 ± 0.37	2163 ± 418
	winter	8.0 ± 3.0	0.6 ± 0.5	0.2 ± 0.1	0.2 ± 0.1	21.5 ± 5.1	1.09 ± 0.64	1057 ± 534
	spring	1.7 ± 1.0	0.5 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	15.1 ± 4.7	2.92 ± 3.63	496 ± 199
	summer	4.5 ± 3.0	2.1 ± 1.6	0.5 ± 0.3	0.6 ± 0.3	45.6 ± 5.8	71.1 ± 64.4	1956 ± 1486
	annual	16.4 ± 2.7	4.2 ± 2.4	1.2 ± 0.3	1.2 ± 0.4	121.8 ± 17.3	76.0 ± 62.4	5671 ± 1373
black locust	autumn	2.5 ± 1.0	2.0 ± 1.3	0.4 ± 0.2	0.4 ± 0.2	32.4 ± 4.5	1.69 ± 1.52	2857 ± 644
	winter	8.3 ± 3.3	1.1 ± 0.3	0.3 ± 0.1	0.3 ± 0.1	21.9 ± 8.2	0.82 ± 0.54	1874 ± 438
	spring	3.9 ± 1.3	1.0 ± 0.9	0.2 ± 0.2	0.3 ± 0.2	14.4 ± 5.6	1.83 ± 3.16	942 ± 694
	summer	6.3 ± 2.2	2.5 ± 1.7	0.6 ± 0.4	0.6 ± 0.4	31.5 ± 12.0	29.7 ± 39.4	2782 ± 1294
	annual	21.0 ± 6.4	6.5 ± 3.5	1.5 ± 0.7	1.6 ± 0.8	100.2 ± 10.0	33.8 ± 37.7	7757 ± 994

Table A.2 (continued)

aspen	autumn	3.3 ± 2.4	1.8 ± 1.1	0.4 ± 0.3	0.3 ± 0.1	22.4 ± 3.5	0.06 ± 0.04	2669 ± 991
	winter	8.5 ± 2.4	2.0 ± 1.3	0.4 ± 0.2	0.4 ± 0.2	15.1 ± 5.3	0.13 ± 0.05	2351 ± 967
	spring	3.3 ± 0.6	1.1 ± 0.6	0.3 ± 0.2	0.3 ± 0.2	12.9 ± 3.2	0.14 ± 0.19	867 ± 216
	summer	9.1 ± 2.8	3.4 ± 1.4	0.9 ± 0.4	0.8 ± 0.3	31.9 ± 4.3	0.26 ± 0.08	2533 ± 391
	annual	24.2 ± 5.4	8.3 ± 3.9	2.0 ± 1.1	1.8 ± 0.8	82.3 ± 9.0	0.6 ± 0.1	8419 ± 1878
Scots pine	autumn	2.2 ± 1.0	2.1 ± 1.0	0.4 ± 0.2	0.4 ± 0.2	31.3 ± 8.4	0.69 ± 0.52	824 ± 192
	winter	5.2 ± 0.6	1.3 ± 0.6	0.3 ± 0.1	0.3 ± 0.1	16.6 ± 6.8	1.74 ± 2.16	629 ± 267
	spring	2.1 ± 0.4	0.9 ± 0.4	0.2 ± 0.1	0.2 ± 0.1	12.0 ± 2.5	1.37 ± 2.17	500 ± 181
	summer	5.7 ± 1.4	3.5 ± 1.1	0.8 ± 0.2	0.8 ± 0.2	46.6 ± 10.1	38.4 ± 18.9	1676 ± 384
	annual	15.2 ± 2.7	6.7 ± 2.1	1.7 ± 0.5	1.7 ± 0.5	125.9 ± 40.7	42.2 ± 17.6	3630 ± 883
Douglas fir	autumn	2.4 ± 2.4	0.8 ± 0.4	0.5 ± 0.6	0.2 ± 0.1	22.3 ± 4.6	7.69 ± 10.9	1380 ± 1101
	winter	5.9 ± 0.7	1.9 ± 1.7	0.2 ± 0.1	0.2 ± 0.1	12.8 ± 3.5	0.37 ± 0.32	1124 ± 394
	spring	1.7 ± 0.7	0.4 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	10.2 ± 1.9	1.60 ± 2.94	609 ± 266
	summer	3.1 ± 1.9	1.3 ± 0.8	0.4 ± 0.1	0.4 ± 0.1	43.9 ± 12.0	6.98 ± 8.02	2347 ± 1131
	annual	13.1 ± 3.2	4.4 ± 1.7	1.1 ± 0.7	0.8 ± 0.3	89.2 ± 17.6	16.6 ± 11.9	5293 ± 2427

§ autumn: 19/09/2009 - 30/11/2009; winter: 01/12/2009 - 31/03/2010; spring: 01/04/2010 - 24/06/2010; summer: 25/06/2010 - 21/09/2010 (see § 5.2.2)

Appendix B

Figures B.1 – B.12 show the calibration and validation results at four depths (5, 15, 30 and 50 cm) under the different tree species, i.e. Hydrus-1D model output (blue line) vs. observed values (red line)

Calibration: March 2011 – February 2012
Validation: March 2012 – February 2013
(See § 7.2.3)

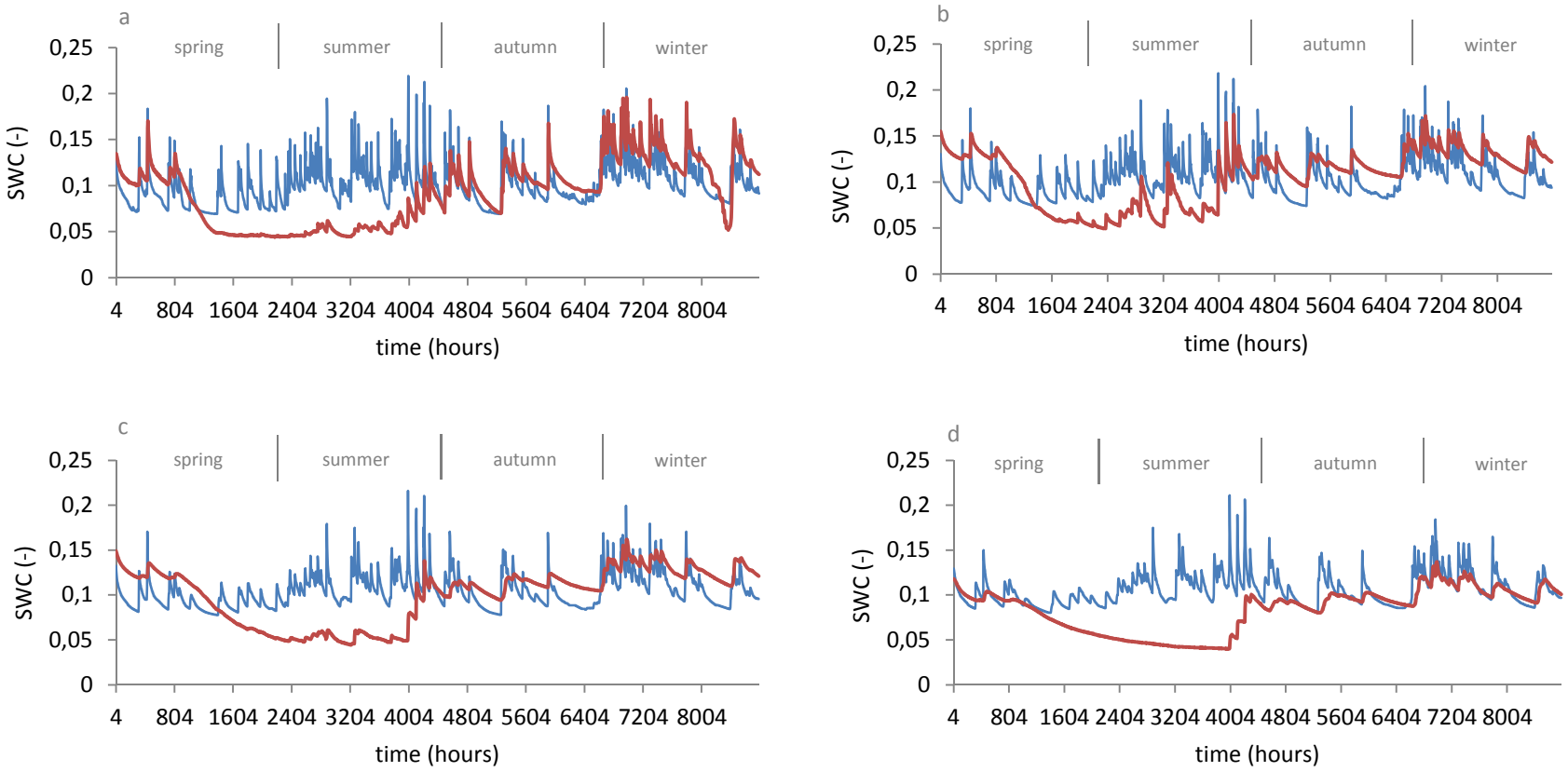


Figure B.1 Calibration results for silver birch at 5 (a), 15 (b), 30 (c) and 50 (d) cm depth (model output vs. observed values)

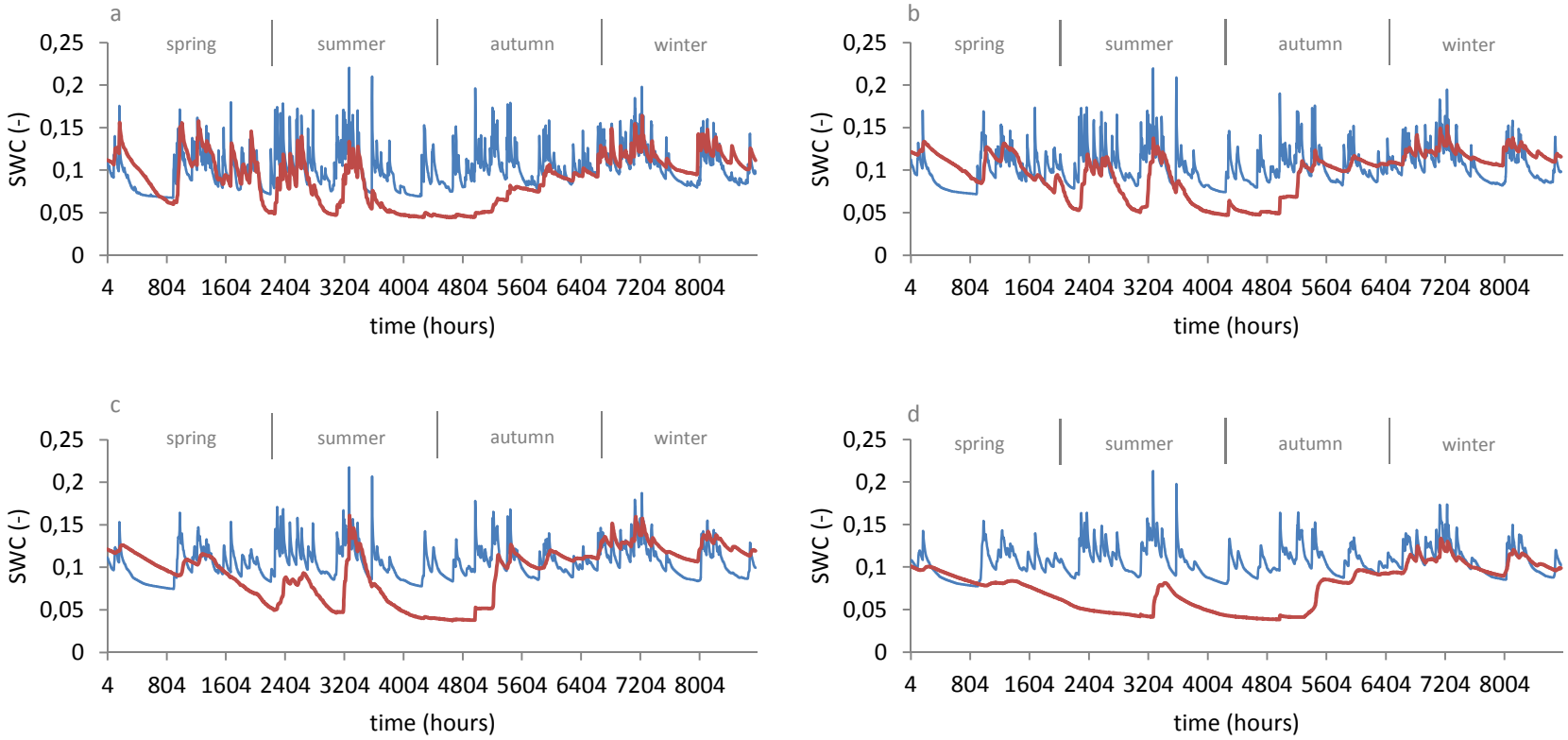
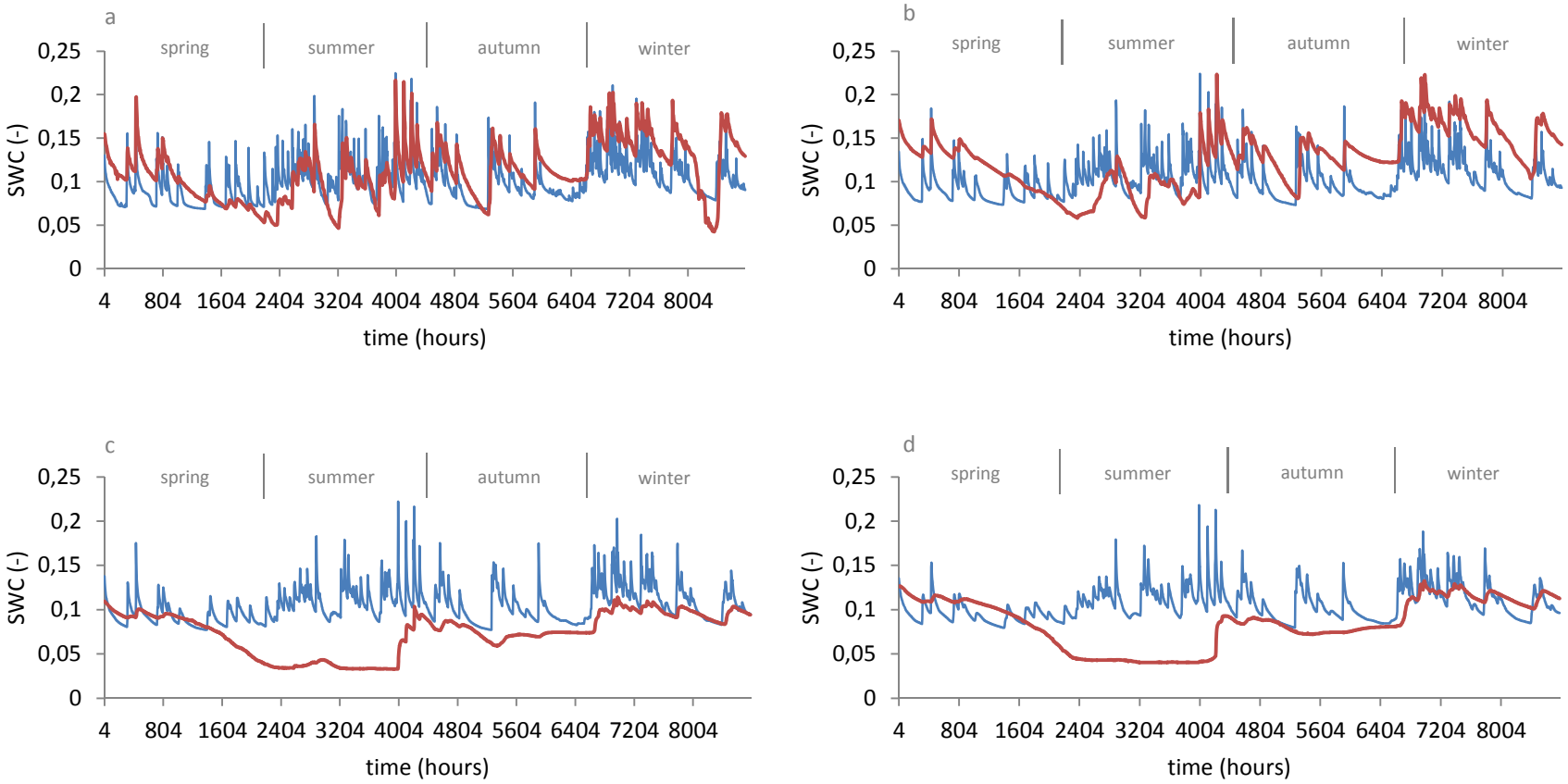


Figure B.2 Validation results for silver birch at 5 (a), 15 (b), 30 (c) and 50 (d) cm depth (model output vs. observed values)



180 **Figure B.3 Calibration results for oak at 5 (a), 15 (b), 30 (c) and 50 (d) cm depth (model output vs. observed values)**

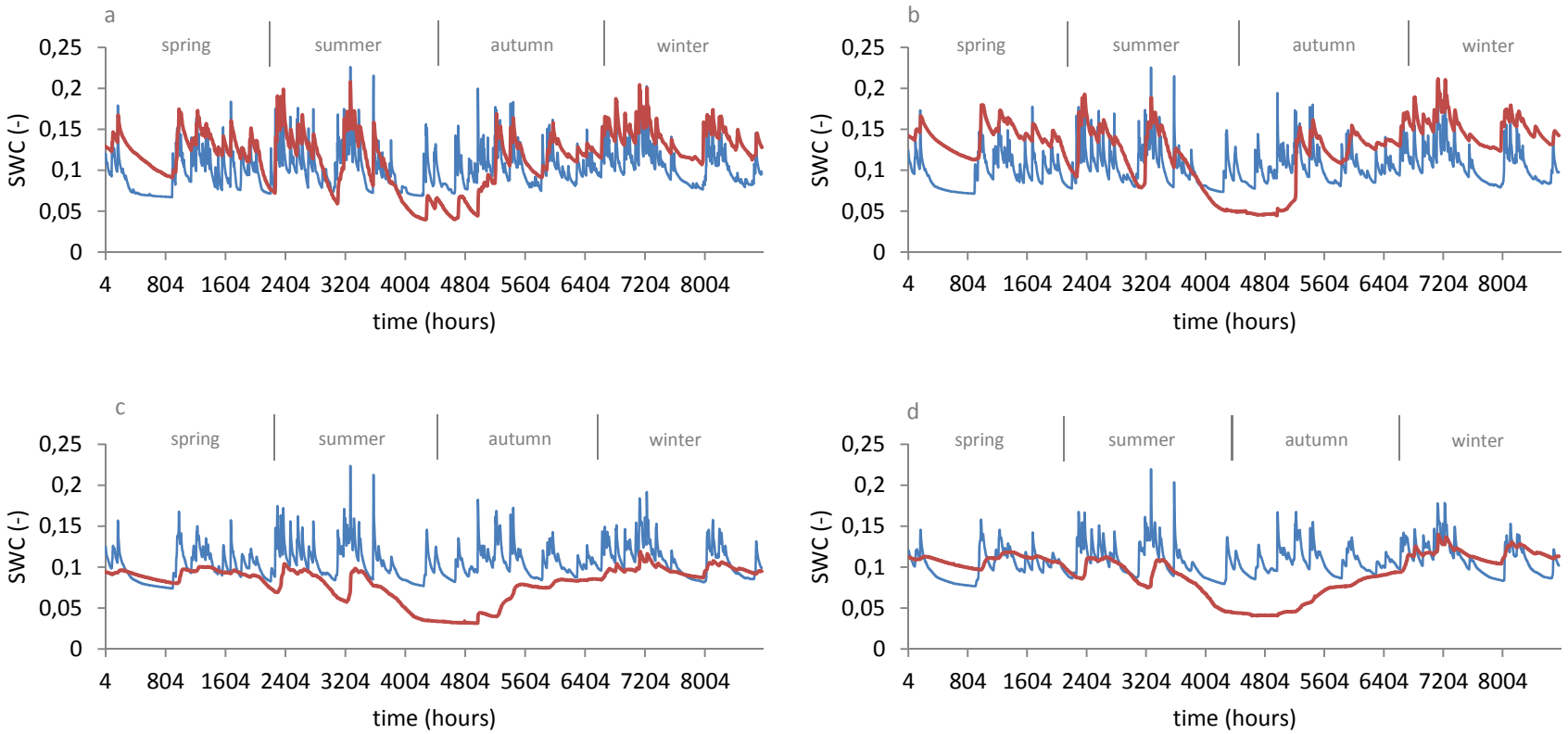


Figure B.4 Validation results for oak at 5 (a), 15 (b), 30 (c) and 50 (d) cm depth (model output vs. observed values)

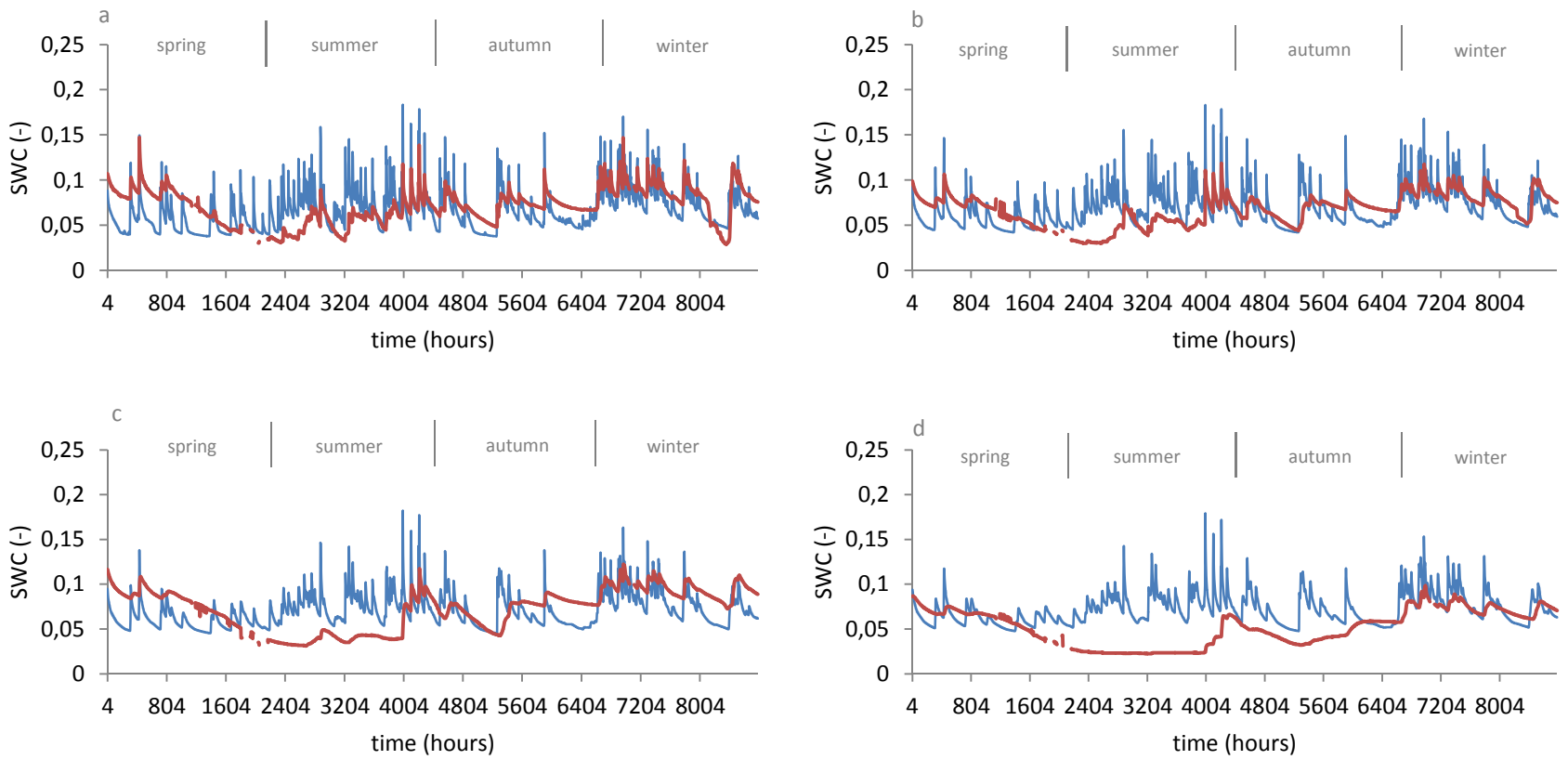


Figure B.5 Calibration results for black locust at 5 (a), 15 (b), 30 (c) and 50 (d) cm depth (model output vs. observed values)

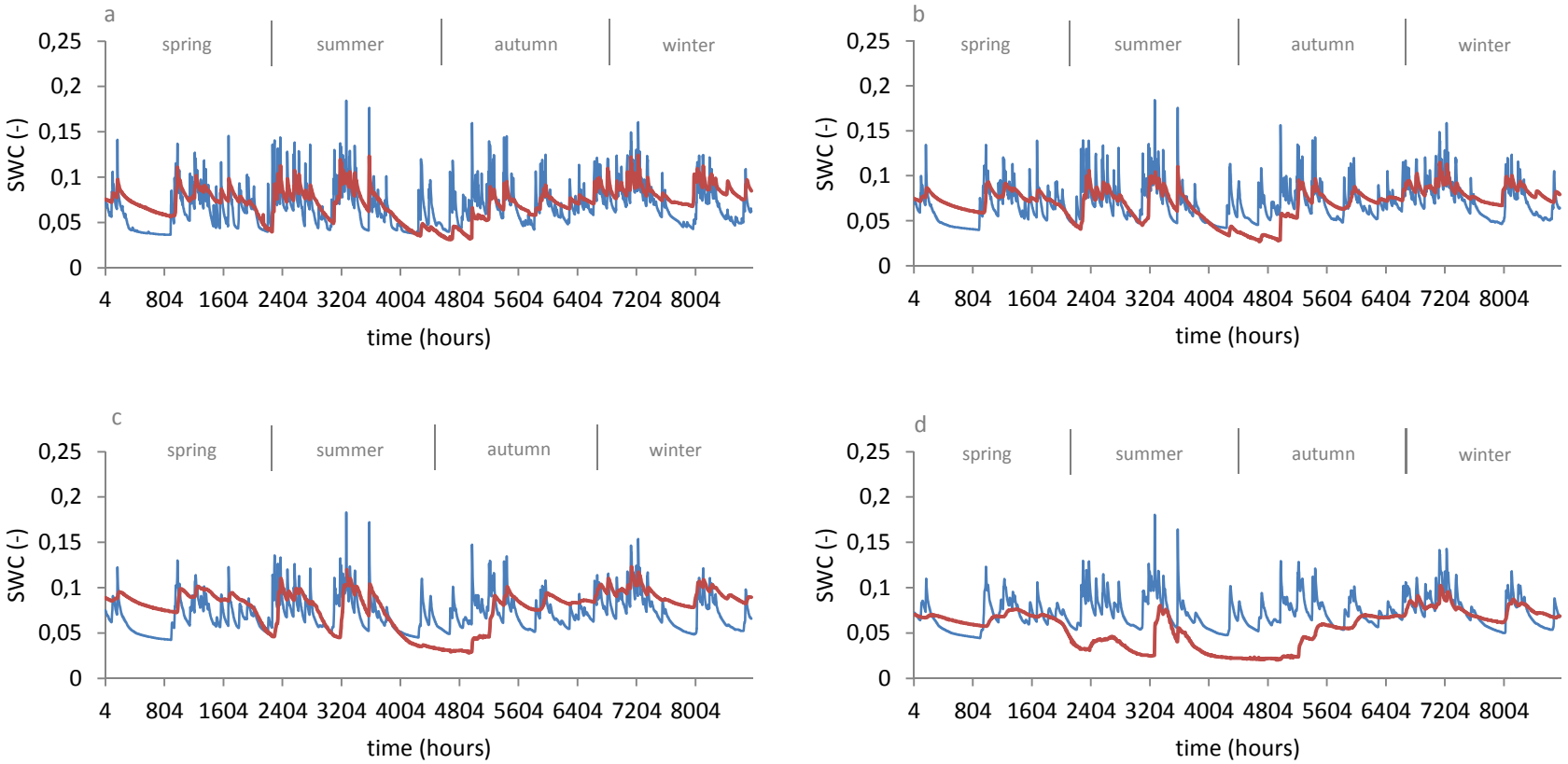


Figure B.6 Validation results for black locust at 5 (a), 15 (b), 30 (c) and 50 (d) cm depth (model output vs. observed values)

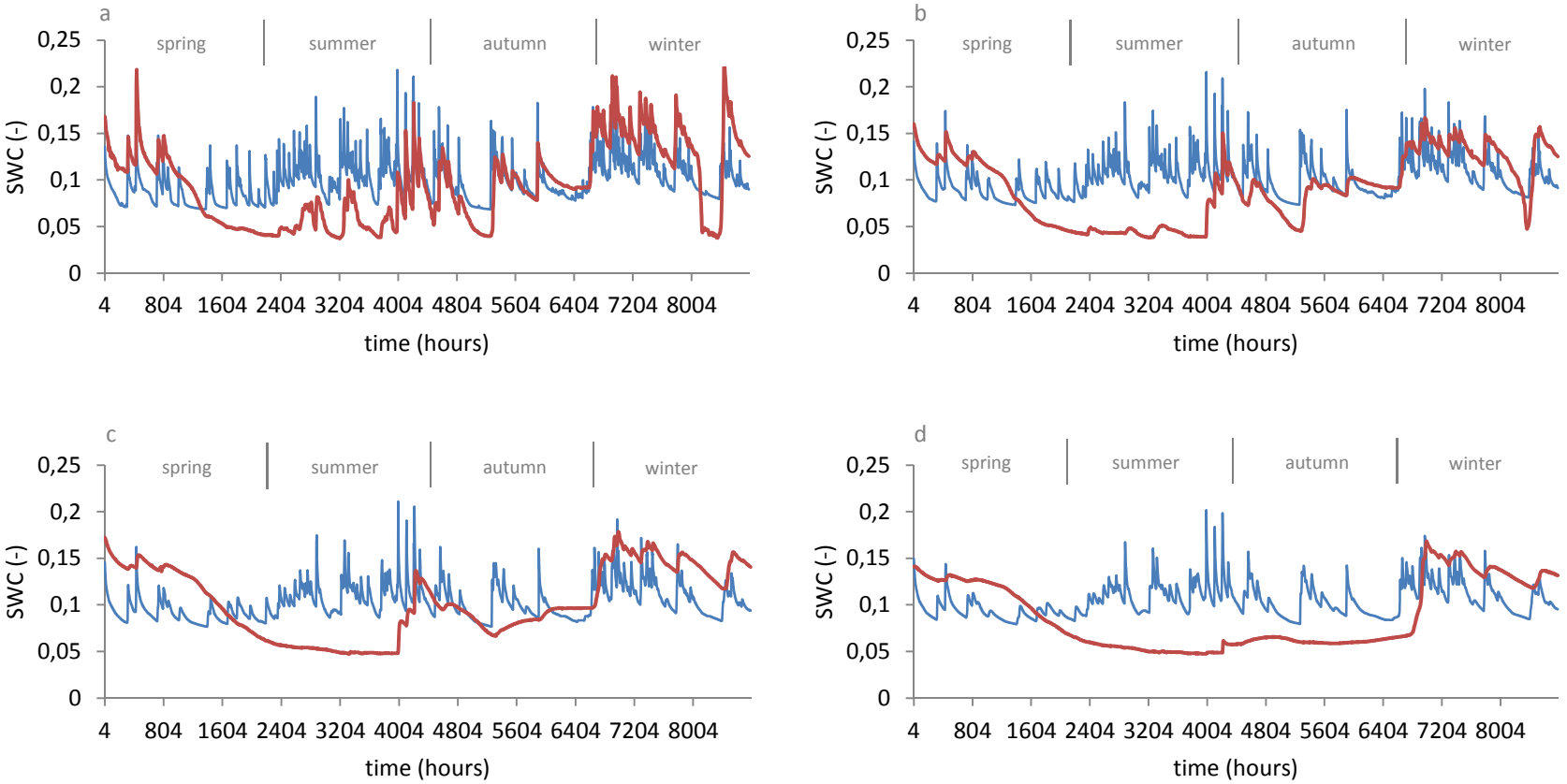


Figure B.7 Calibration results for aspen at 5 (a), 15 (b), 30 (c) and 50 (d) cm depth (model output vs. observed values)

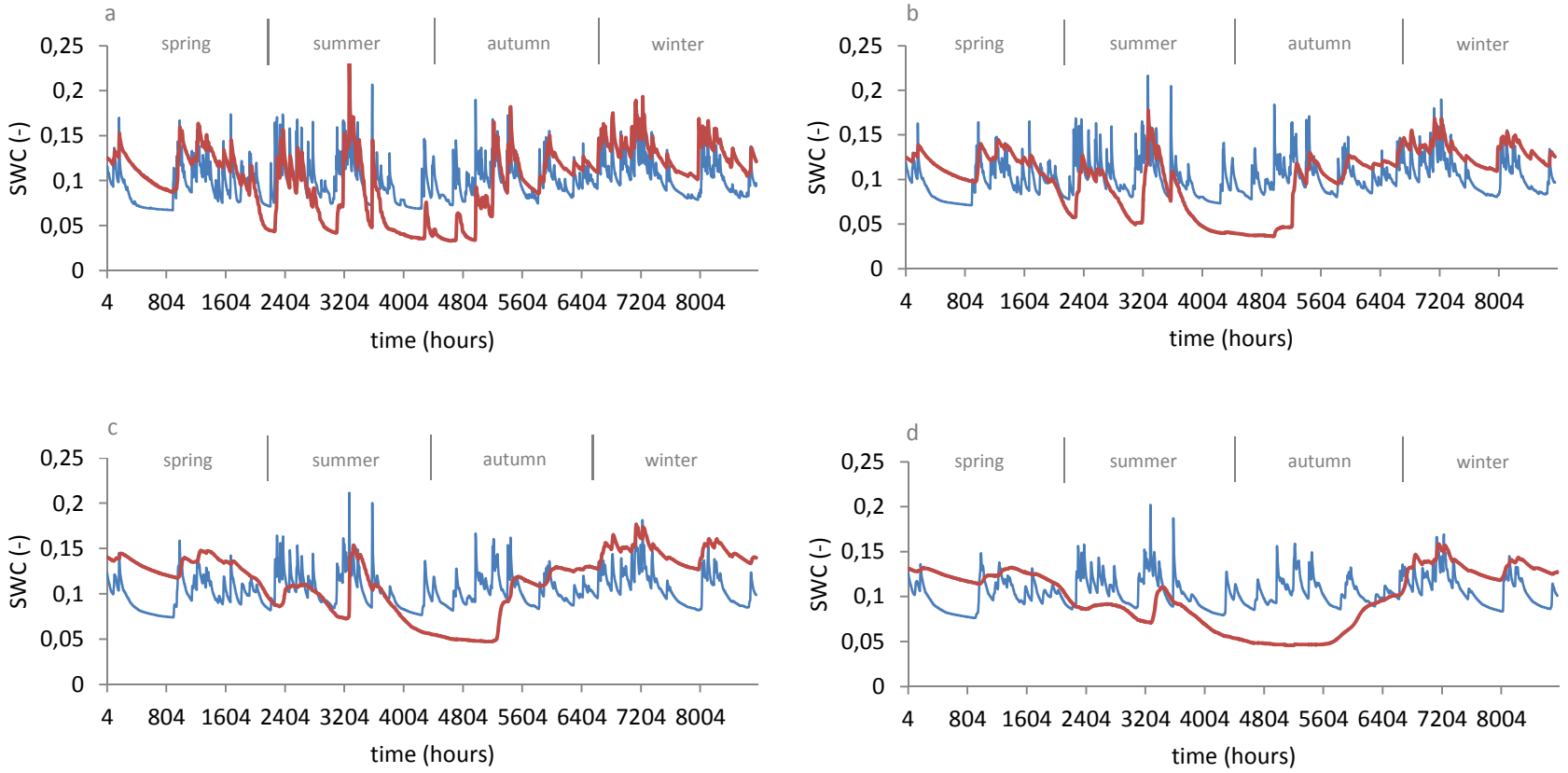


Figure B.8 Validation results for aspen at 5 (a), 15 (b), 30 (c) and 50 (d) cm depth (model output vs. observed values)

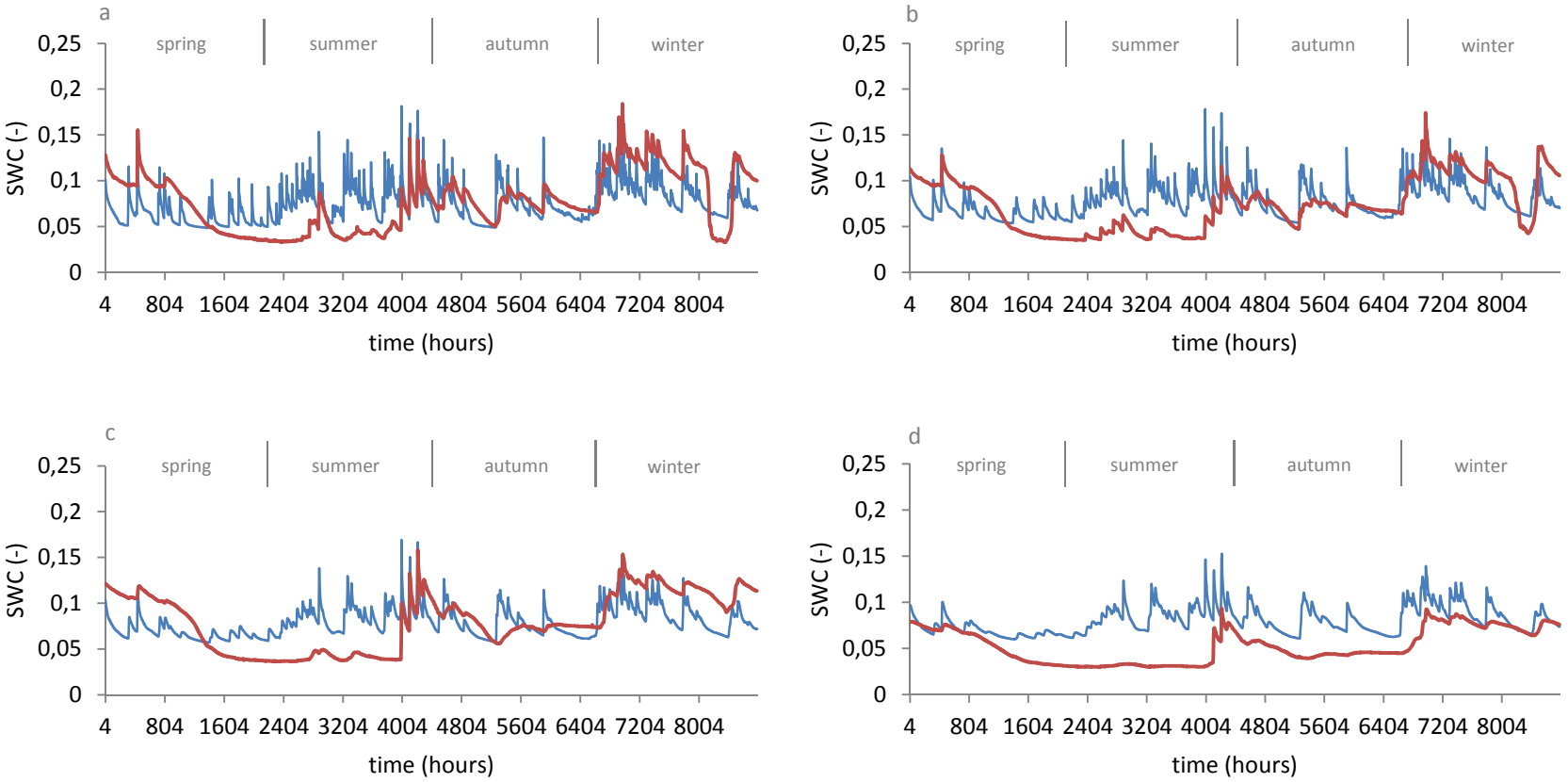


Figure B.9 Calibration results for Scots pine at 5 (a), 15 (b), 30 (c) and 50 (d) cm depth (model output vs. observed values)

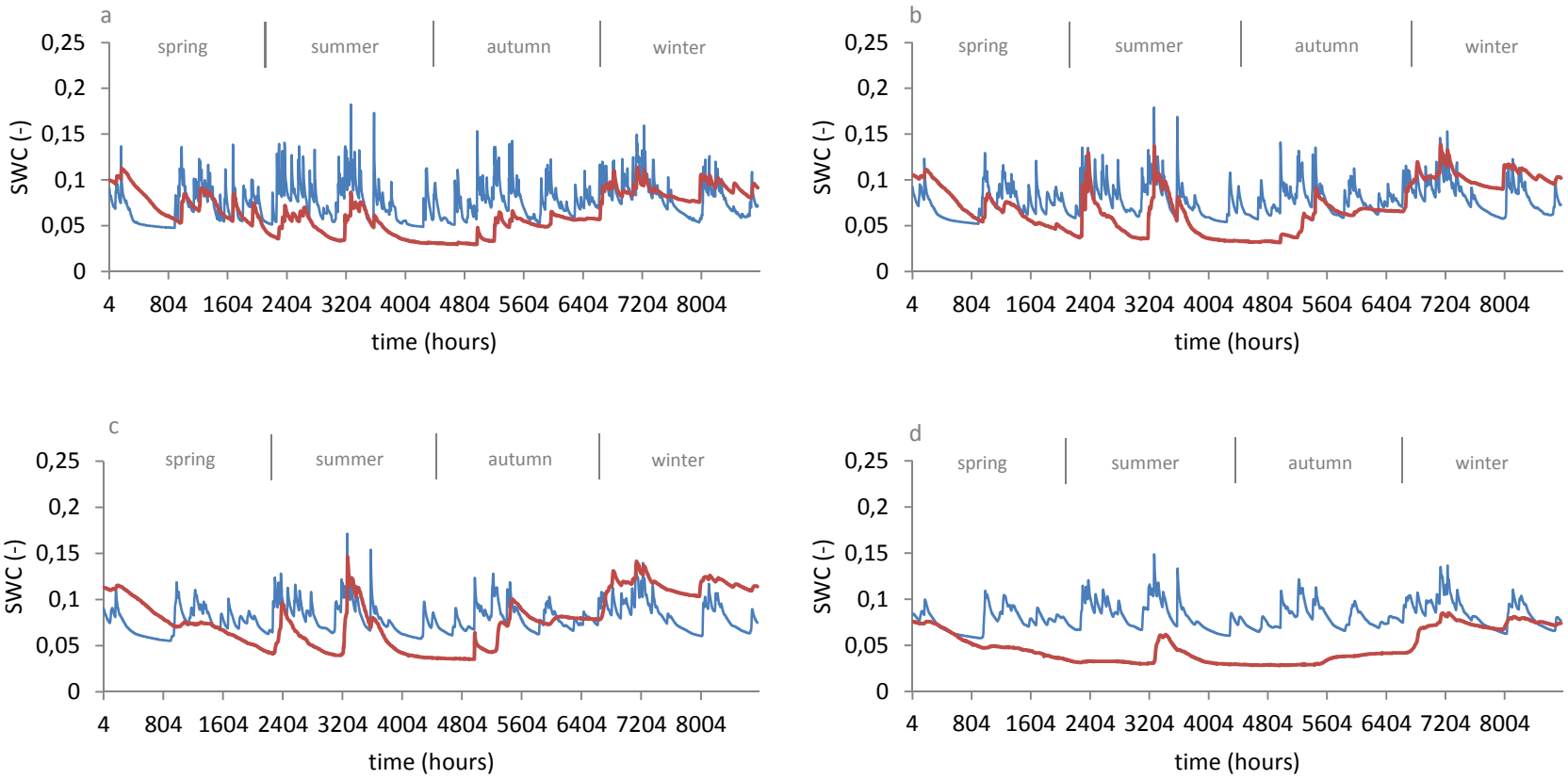


Figure B.10 Validation results for Scots pine at 5 (a), 15 (b), 30 (c) and 50 (d) cm depth (model output vs. observed values)

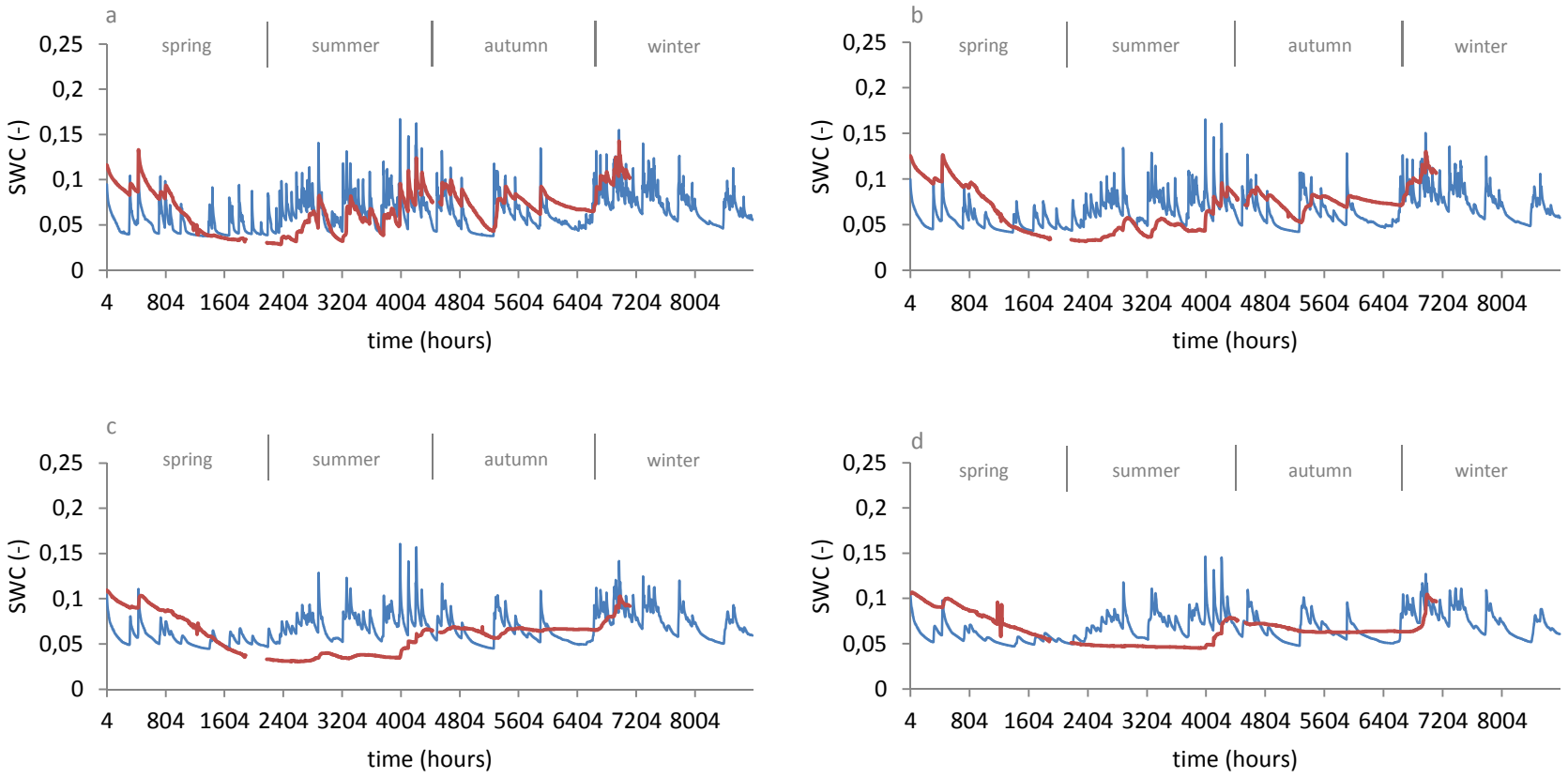


Figure B.11 Calibration results for Douglas fir at 5 (a), 15 (b), 30 (c) and 50 (d) cm depth (model output vs. observed values)

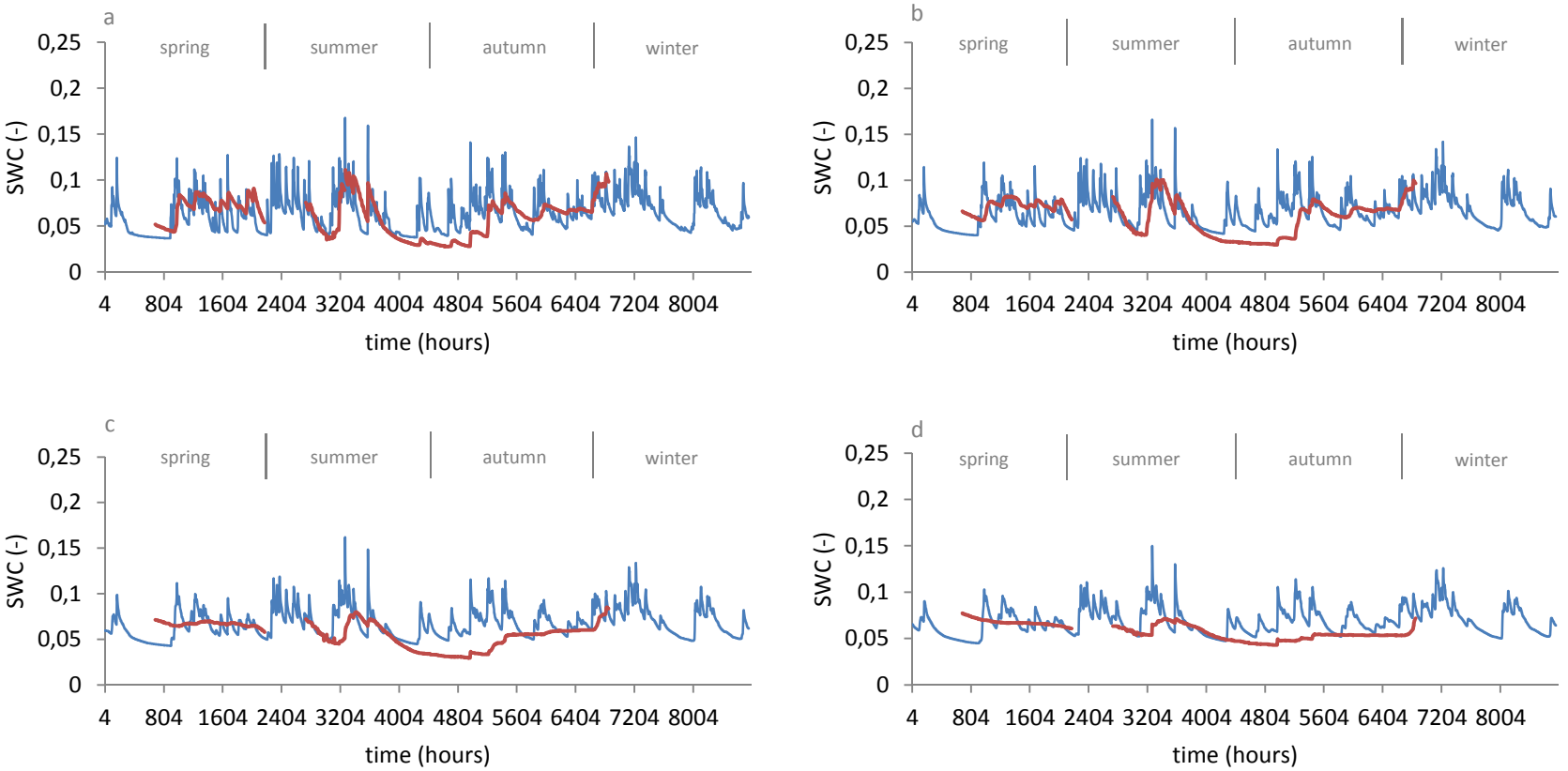


Figure B.12 Validation results for Douglas fir at 5 (a), 15 (b), 30 (c) and 50 (d) cm depth (model output vs. observed values)

Appendix C

Table C.1 Seasonal pH and concentrations of Cd and Zn (total and dissolved), DOC, base cations (BC) and anions in the soil solution at 50 cm depth under the six tree species (n = 6) (average ± st.dev)

	season §	pH (-)	Cd_{tot} ($\mu g\ l^{-1}$)	Cd_{diss} ($\mu g\ l^{-1}$)	Zn_{tot} ($mg\ l^{-1}$)	Zn_{diss} ($mg\ l^{-1}$)	DOC ($mg\ l^{-1}$)	BC ($meq\ l^{-1}$)	Anions ($meq\ l^{-1}$)
silver birch	winter	5.60 ± 0.17	2.0 ± 1.1	2.3 ± 0.9	0.54 ± 0.24	0.53 ± 0.22	32.4 ± 13.3	0.76 ± 0.27	0.56 ± 0.26
	spring	5.50 ± 0.09	4.0 ± 0.7	2.2 ± 0.8	0.41 ± 0.11	0.41 ± 0.12	27.8 ± 10.2	0.58 ± 0.26	0.38 ± 0.26
	summer	/	/	/	/	/	/	/	/
	autumn	5.60 ± 0.17	3.1 ± 1.2	3.3 ± 0.7	0.50 ± 0.08	0.60 ± 0.25	38.9 ± 13.6	1.04 ± 0.44	0.66 ± 0.24
oak	winter	5.90 ± 0.47	2.5 ± 1.6	2.6 ± 1.0	0.52 ± 0.07	0.52 ± 0.08	49.1 ± 28.4	0.81 ± 0.25	0.51 ± 0.16
	spring	6.22 ± 0.54	1.3 ± 1.1	1.8 ± 0.4	0.36 ± 0.04	0.36 ± 0.04	32.7 ± 15.6	0.64 ± 0.29	0.32 ± 0.10
	summer	/	/	/	/	/	/	/	/
	autumn	6.07 ± 0.73	4.3 ± 2.1	3.1 ± 1.7	0.59 ± 0.16	0.57 ± 0.16	50.2 ± 19.9	0.98 ± 0.23	0.53 ± 0.10
black locust	winter	5.21 ± 0.14	6.1 ± 2.3	6.4 ± 2.8	1.42 ± 0.61	1.51 ± 0.66	23.9 ± 7.6	2.30 ± 1.07	2.35 ± 1.30
	spring	5.71 ± 0.37	3.0 ± 0.7	4.3 ± 2.3	0.70 ± 0.03	1.08 ± 0.72	16.6 ± 4.0	1.52 ± 0.47	1.46 ± 0.53
	summer	/	/	/	/	/	/	/	/
	autumn	5.60 ± 0.16	5.3 ± 0.9	4.2 ± 0.6	0.84 ± 0.14	0.81 ± 0.14	28.9 ± 10.1	1.13 ± 0.27	0.94 ± 0.34

Table C.1 (continued)

aspen	winter	5.75 ± 0.22	3.6 ± 1.1	3.7 ± 1.4	0.60 ± 0.25	0.64 ± 0.27	25.0 ± 5.0	1.10 ± 0.46	0.90 ± 0.47
	spring	5.85 ± 0.24	3.6 ± 1.6	2.9 ± 0.9	0.46 ± 0.18	0.44 ± 0.18	23.0 ± 7.6	0.74 ± 0.23	0.58 ± 0.27
	summer	/	/	/	/	/	/	/	/
	autumn	5.80 ± 0.19	5.6 ± 1.2	4.8 ± 0.9	0.66 ± 0.21	0.72 ± 0.29	37.6 ± 6.7	1.15 ± 0.16	0.82 ± 0.15
Scots pine	winter	5.84 ± 0.90	9.3 ± 4.6	10.2 ± 5.8	2.61 ± 2.08	3.00 ± 2.28	38.2 ± 18.1	2.91 ± 0.84	2.75 ± 1.02
	spring	5.98 ± 0.61	4.1	8.2 ± 6.3	0.62	2.83 ± 3.15	48.0	2.23 ± 0.29	1.53
	summer	/	/	/	/	/	/	/	/
	autumn	5.79 ± 0.65	15.2 ± 16.1	18.0 ± 13.3	1.88 ± 1.99	3.85 ± 3.66	78.4 ± 60.5	2.56 ± 1.80	3.28 ± 2.21
Douglas fir	winter	5.00 ± 0.54	6.9 ± 4.7	6.1 ± 3.2	1.08 ± 0.66	1.07 ± 0.63	33.9 ± 7.6	2.02 ± 1.06	1.79 ± 1.07
	spring	5.11 ± 0.26	7.9 ± 2.8	6.2 ± 5.2	0.95 ± 0.77	0.87 ± 0.82	27.8 ± 3.5	1.84 ± 0.85	1.42 ± 1.05
	summer	/	/	/	/	/	/	/	/
	autumn	5.45 ± 0.23	16.6	10.5 ± 9.3	2.19	1.32 ± 1.17	33.4 ± 2.9	2.71 ± 1.30	2.67 ± 1.67

[§] winter: 23/11/2009 - 31/03/2010; spring: 01/04/2010 - 25/06/2010; summer: 26/06/2010 - 21/09/2010; autumn: 22/09/2010 - 25/11/2010 (see § 7.2.2)

Table C.2 Seasonal and annual seepage fluxes of Cd and Zn (total and dissolved), DOC, H^+, base cations (BC) and anions at 50 cm depth under the six tree species (n = 6) (average ± st.dev)

	season §	Cd_{tot} (g ha^{-1} yr^{-1})	Cd_{diss} (g ha^{-1} yr^{-1})	Zn_{tot} (kg ha^{-1} yr^{-1})	Zn_{diss} (kg ha^{-1} yr^{-1})	DOC (kg ha^{-1} yr^{-1})	H^+ (mol ha^{-1} yr^{-1})	BC (eq ha^{-1} yr^{-1})	Anions (eq ha^{-1} yr^{-1})
silver birch	winter	5.2 ± 2.3	6.2 ± 1.7	1.5 ± 0.5	1.4 ± 0.5	85.9 ± 19.2	7.44 ± 2.17	2042 ± 570	1494 ± 552
	spring	1.9 ± 0.2	1.0 ± 0.3	0.2 ± 0.0	0.2 ± 0.0	13.3 ± 4.4	1.55 ± 0.26	278 ± 124	183 ± 111
	summer	/	/	/	/	/	/	/	/
	autumn	5.3 ± 1.5	5.5 ± 1.1	0.8 ± 0.1	1.0 ± 0.4	65.2 ± 17.7	4.49 ± 1.36	1747 ± 731	1109 ± 309
	annual	12.4 ± 3.0	12.7 ± 2.8	2.5 ± 0.6	2.6 ± 0.6	164.4 ± 45.2	13.5 ± 3.8	4067 ± 1250	2786 ± 945
oak	winter	7.3 ± 3.6	7.4 ± 1.4	1.5 ± 0.1	1.5 ± 0.2	138.7 ± 44.2	5.42 ± 3.31	2371 ± 493	1492 ± 306
	spring	0.6 ± 0.4	0.9 ± 0.2	0.2 ± 0.0	0.2 ± 0.0	15.7 ± 6.1	0.44 ± 0.34	307 ± 114	156 ± 40
	summer	/	/	/	/	/	/	/	/
	autumn	7.7 ± 3.0	5.6 ± 2.5	1.1 ± 0.2	1.0 ± 0.2	90.5 ± 29.4	3.05 ± 2.76	1768 ± 342	955 ± 146
	annual	15.6 ± 6.7	13.9 ± 4.0	2.8 ± 0.4	2.8 ± 0.4	244.9 ± 84.3	8.91 ± 6.85	4445 ± 869	2603 ± 493
black locust	winter	17.7 ± 4.0	18.1 ± 3.9	4.1 ± 1.1	4.3 ± 1.1	67.9 ± 5.2	19.1 ± 4.82	6538 ± 1552	6628 ± 1743
	spring	1.3 ± 0.2	1.9 ± 1.0	0.3 ± 0.0	0.5 ± 0.3	7.4 ± 1.8	1.13 ± 0.81	677 ± 211	649 ± 237
	summer	/	/	/	/	/	/	/	/
	autumn	9.6 ± 1.4	7.6 ± 1.0	1.5 ± 0.2	1.5 ± 0.2	52.2 ± 16.3	4.75 ± 1.56	2031 ± 432	1688 ± 552
	annual	28.7 ± 4.8	27.6 ± 4.9	5.9 ± 1.4	6.2 ± 1.5	127.5 ± 21.8	25.0 ± 7.9	9247 ± 2237	8965 ± 2344

Table C.2 (continued)

aspen	winter	8.5 ± 2.3	8.7 ± 2.8	1.4 ± 0.5	1.5 ± 0.5	58.4 ± 6.8	4.82 ± 2.04	2570 ± 921	2095 ± 918
	spring	1.3 ± 0.5	1.1 ± 0.3	0.2 ± 0.1	0.2 ± 0.1	8.3 ± 2.5	0.59 ± 0.32	269 ± 83	208 ± 88
	summer	/	/	/	/	/	/	/	/
	autumn	8.1 ± 1.6	6.9 ± 1.3	1.0 ± 0.3	1.0 ± 0.4	54.7 ± 9.8	2.48 ± 0.83	1669 ± 233	1195 ± 216
	annual	17.9 ± 3.2	16.7 ± 4.4	2.5 ± 0.9	2.7 ± 1.0	121.4 ± 15.5	7.89 ± 2.66	4508 ± 1356	3498 ± 1314
Scots pine	winter	12.4 ± 4.8	13.2 ± 5.8	3.4 ± 2.0	3.9 ± 2.0	49.6 ± 19.5	4.82 ± 5.58	3775 ± 906	3546 ± 1033
	spring	0.6 ± 0.0	1.2 ± 0.4	0.1 ± 0.0	0.4 ± 0.2	7.2 ± 0.0	0.24 ± 0.12	336 ± 27	229 ± 0
	summer	/	/	/	/	/	/	/	/
	autumn	12.4 ± 5.9	14.7 ± 6.8	1.5 ± 0.7	2.5 ± 1.2	64.0 ± 31.2	2.47 ± 1.79	3110 ± 1299	2678 ± 1142
	annual	25.4 ± 9.6	29.1 ± 12.6	5.1 ± 2.6	5.9 ± 2.7	120.8 ± 50.1	6.79 ± 6.22	7220 ± 2176	6453 ± 2119
Douglas fir	winter	13.1 ± 5.3	11.0 ± 4.3	2.0 ± 0.8	1.9 ± 0.9	59.9 ± 8.9	41.4 ± 38.1	3648 ± 1418	3221 ± 1454
	spring	1.4 ± 0.2	1.1 ± 0.4	0.2 ± 0.1	0.2 ± 0.1	5.0 ± 0.3	1.51 ± 0.38	329 ± 96	254 ± 84
	summer	/	/	/	/	/	/	/	/
	autumn	17.7 ± 0.0	11.2 ± 4.4	2.3 ± 0.0	1.4 ± 0.6	35.6 ± 1.4	4.08 ± 0.94	2891 ± 620	2843 ± 794
	annual	32.2 ± 6.0	23.3 ± 9.0	4.5 ± 0.9	3.5 ± 1.4	100.4 ± 11.1	47.0 ± 39.0	6868 ± 1806	6318 ± 1957

§ winter: 23/11/2009 - 31/03/2010; spring: 01/04/2010 - 25/06/2010; summer: 26/06/2010 - 21/09/2010; autumn: 22/09/2010 - 25/11/2010 (see § 7.2.2)

References

Aber, J.D., Melillo, J.M., 1982. Nitrogen immobilization in decaying hardwood leaf litter as a function of initial nitrogen and lignin content. Can. J. Bot. 60, 2263-2269.

Adair, E.C., Parton, W.J., Del Grosso, S.J., Silver, W.L., Harmon, M.E., Hall, S.A., Burke, I.C., Hart, S.C., 2008. Simple three-pool model accurately describes patterns of long-term litter decomposition in diverse climates. Glob. Change Biol. 14, 2636-2660.

Aerts, R., 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. Oikos 79, 439-449.

Aerts, R., Chapin, F.S., 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv. Ecol. Res. 30, 1-67.

Ahokas, H., 1997. Acidification of forest top soils in 60 years to the southwest of Helsinki. For. Ecol. Manag. 94, 187-193.

Akselsson, C., Hultberg, H., Karlsson, P.E., Karlsson, G.P., Hellsten, S., 2013. Acidification trends in south Swedish forest soils 1986-2008 - Slow recovery and high sensitivity to sea-salt episodes. Sci. Total Environ. 444, 271-287.

Alcock, M.R., Morton, A.J., 1985. Nutrient content of throughfall and stemflow in woodland recently established on heathland. J. Ecol. 73, 625-632.

Aldenberg, T., Slob, W., 1993. Confidence limits for hazardous concentrations based on logistically distributed NOEC toxicity data. Ecotox. Environ. Safe. 25, 48-63.

Alriksson, A., Eriksson, H.M., 1998. Variations in mineral nutrient and C distribution in the soil and vegetation compartments of five temperate tree species in NE Sweden. For. Ecol. Manag. 108, 261-273.

Alriksson, A., Eriksson, H.M., 2001. Distribution of Cd, Cu, Pb and Zn in soil and vegetation compartments in stands of five boreal tree species in N.E. Sweden. Water Air Soil Pollut. Focus 1, 461-475.

Andersen, M.K., Refsgaard, A., Raulund-Rasmussen, K., Strobel, B.W., Hansen, H.C.B., 2002. Content, distribution, and solubility of cadmium in arable and forest soils. Soil Sci. Soc. Am. J. 66, 1829-1835.

Angle, J.S., Linacre, N.A., 2005. Metal phytoextraction - a survey of potential risks. Int. J. Phytoremediat. 7, 241-254.

Aubert, M., Margerie, P., Ernoult, A., Decaëns, T., Bureau, F., 2006. Variability and heterogeneity of humus forms at stand level: comparison between pure beech and mixed beech-hornbeam forest. Ann. For. Sci. 63, 177-188.

Audet, P., Charest, C., 2007. Heavy metal phytoremediation from a meta-analytical perspective. Environ. Pollut. 147, 231-237.

Augusto, L., De Schrijver, A., Vesterdal, L., Smolander, A., Prescott, C., Ranger, J., 2014. Influences of evergreen gymnosperm and deciduous angiosperm tree species on the functioning of temperate and boreal forests. Biol. Rev., in press.

Augusto, L., Ranger, J., Binkley, D., Rothe, A., 2002. Impact of several common tree species of European temperate forests on soil fertility. Ann. For. Sci. 59, 233-253.

Azevedo, L.B., van Zelm, R., Hendriks, A.J., Bobbink, R., Huijbregts, M.A.J., 2013. Global assessment of the effects of terrestrial acidification on plant species richness. Environ. Pollut. 174, 10-15.

Baker, A.J.M., Brooks, R.R., 1989. Terrestrial higher plants which hyperaccumulate metallic elements - a review of their distribution, ecology and phytochemistry. Biorecovery 1, 81-126.

Baker, A.J.M., McGrath, S.P., Reeves, R.D., Smith, J.A.C., 2000. Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metalpolluted soils. In: Terry, N., Bañuelos, G. (Eds) Phytoremediation of contaminated soil and water. Lewis, Boca Raton, pp 85-108.

Bates, D., Maechler, M., Bolker, B., 2011. lme4: linear mixed-effects models using S4 classes. R package version 0.999375-38. http://CRAN.R-project.org/package=lme4.

Becker, R.H., Zmijewski, K.A., Crail, T., 2013. Seeing the forest for the invasives: mapping buckthorn in the oak openings. Biol. Invasions 15, 315-326.

Behmer, S.T., Lloyd, C.M., Raubenheimer, D., Stewart-Clark, J., Knight, J., Leighton, R.S., Harper, F.A., Smith, J.A.C., 2005. Metal hyperaccumulation in plants: mechanisms of defence against insect herbivores. Funct. Ecol. 19, 55-66.

Benjamin, M. M., Leckie, J.O., 1982. Effects of complexation by Cl, SO_4, and S_2O_3 on adsorption behavior of Cd on oxide surfaces. Environ. Sci. Technol. 16, 162-170.

Berg, B., 2000. Litter decomposition and organic matter turnover in northern forest soils. For. Ecol. Manag. 133, 13-22.

Berg, B., Berg, M.P., Bottner, P., Box, E., Breymeyer, A., Calvo De Anta, R., Coûteaux, M., Escudero, A., Gallardo, A., Kratz, W., Madeira, M., Mälkönen, E., McClaugherty, C., Meentemeyer, V., Munoz, F., Piussi, P., Remacle, J., Virzo de Santo, A., 1993. Litter mass loss rates in pine forests of Europe and Eastern United States: some relationships with climate and litter quality. Biogeochemistry 20, 127-159.

Berg, B., Ekbohm, G., Söderström, B., Staaf, H., 1991. Reduction of decomposition rates of Scots pine needle litter due to heavy metal pollution. Water Air Soil Pollut. 59, 165-177.

Berg, B., McClaugherty, C., Virzo De Santo, A., Johnson, D., 2001. Humus buildup in boreal forests: effects of litter fall and its N concentration. Can. J. For. Res. 31, 988-998.

Berg, B., Staaf, H., 1981. Leaching, accumulation and release of nitrogen in decomposing forest litter. In: Clark, F.E., Rosswall, T. (Eds) Terrestrial Nitrogen Cycles. Ecol. Bull. 33, Stockholm, pp 163-178.

Berg, B., Staaf, H., 1987. Release of nutrients from decomposing white birch leaves and Scots pine needle litter. Pedobiologia 30, 55-63.

Bergholm, J., Berggren, D., Alavi, G., 2003. Soil acidification induced by ammonium sulphate addition in a Norway spruce forest in southwest Sweden. Water Air Soil Pollut. 148, 87-109.

Bergkvist, B., Folkeson, L., 1995. The influence of tree species on acid deposition, proton budgets and element fluxes in south Swedish forest ecosystems. Ecological Bulletin 44, 90-99.

Bergkvist, B., Folkeson, L., Berggren, D., 1989. Fluxes of Cu, Zn, Pb, Cr and Ni in temperate forest ecosystems - a literature review. Water Air Soil Pollut. 47, 217-286.

Berthelsen, B.O., Olsen, R.A., Steinnes, E., 1995. Ectomycorrhizal heavy metal accumulation as a contributing factor to heavy metal levels in organic surface soils. Sci. Total Environ. 170, 141-149.

Berthelsen, B.O., Årdal, L., Steinnes, E., Abrahamsen, G., Stuanes, A.O., 1994. Mobility of heavy metals in pine forest soils as influenced by experimental acidification. Water Air Soil Pollut. 73, 29-48.

Bittner, S., Talkner, U., Krämer, I., Beese, F., Hölscher, D., Priesack, E., 2010. Modeling stand water budgets of mixed temperate broad-leaved forest stands by considering variations in species specific drought response. Agr. Forest Meteorol. 150, 1347-1357.

Blair, J.M., 1988a. Nutrient release from decomposing foliar litter of three tree species with special reference to calcium, magnesium and potassium dynamics. Plant Soil 110, 49-55.

Blair, J.M., 1988b. Nitrogen, sulfur and phosphorus dynamics in decomposing deciduous leaf litter in the southern Appalachians. Soil Biol. Biochem. 20, 693-701.

Boekhold, A.E., Temminghoff, E.J.M., Van der Zee, S.E.A.T.M., 1993. Influence of electrolyte composition and pH on cadmium sorption by an acid sandy soil. Eur. J. Soil Sci. 44, 85-96.

Boring, L.R., Swank, W.T., 1984. The role of black locust (*Robinia pseudoacacia*) in forest succession. J. Ecol. 72, 749-766.

Bouwman, L.A., Bloem, J., Römkens, P.F.A.M., Japenga, J., 2005. EDGA amendment of slightly heavy metal loaded soil affects heavy metal solubility, crop growth and microbivorous nematodes but not bacteria and herbivorous nematodes. Soil Biol. Biochem. 37, 271-278.

Bowman, W.D., Cleveland, C.C., Halada, L., Hresko, J., Baron, J.S., 2008. Negative impact of nitrogen deposition on soil buffering capacity. Nature Geosci. 1, 767-770.

Boyd, R.S., Martens, S.N., 1992. The raison d'être for metal hyperaccumulation by plants. In: Baker, A.J.M., Proctor, J., Reeves, R.D. (Eds) The Vegetation of Ultramafic (Serpentine) Soils. Intercept, Andover, UK, pp 279-289.

Brandtberg, P.O., Lundkvist, H., Bengtsson, J., 2000. Changes in forest-floor chemistry caused by a birch admixture in Norway spruce stands. For. Ecol. Manag. 130, 253-264.

Brekken, A., Steinnes, E., 2004. Seasonal concentrations of cadmium and zinc in native pasture plants: consequences for grazing animals. Sci. Total Environ. 326, 181-195.

Brun, C.B., Åström, M.E., Peltola, P., Johansson, M.B., 2008. Trends in major and trace elements in decomposing needle litters during a long-term experiment in Swedish forests. Plant Soil 306, 199-210.

Buchet, J.P., Lauwerys, R., Roels, H., Bernard, A., Bruaux, P., Claeys, F., Ducoffre, G., de Plaen, P., Staessen, J., Amery, A., Lijnen, P., Thijs, L., Rondia, D., Sartor, F., Saint Remy, A., Nick, L., 1990. Renal effects of cadmium body burden of the general population. Lancet 336, 699-702.

Carnol, M., Bazgir, M., 2013. Nutrient return to the forest floor through litter and throughfall under 7 forest species after conversion from Norway spruce. For. Ecol. Manag. 309, 66-75.

Cavallaro, N., McBride, M.B., 1978. Copper and cadmium adsorption characteristics of selected acid and calcareous soils. Soil Sci. Soc. Am. J. 42, 550-556.

Ceenaeme, J., Geysen, D., Gommeren, E., Dedecker, D., De Naeyer, F., Dries, V., Van Dijck, W., Van Dyck, E., Stalpaert, L., 2004. Bodemverontreiniging. In: MIRA-T 2004. Milieu- en

natuurrapport Vlaanderen: thema's. Leuven, Vlaamse Milieumaatschappij en Garant uitgevers nv, 327-334.

Chaney, R.L., Li, Y.M., Brown, S.L., Homer, F.A., Malik, M., Angle, J.S., Baker, A.J.M., Reeves, R.D., Chin, M., 2000. Improving metal hyperaccumulator wild plants to develop commercial phytoextraction systems: approaches and progress. In: Terry, N., Bañuelos, G. (Eds) Phytoremediation of contaminated soil and water. Lewis, Boca Raton, pp 131-160.

Christensen, T.H., 1984. Cadmium soil sorption at low concentrations: I. Effect of time, cadmium load, pH, and calcium. Water Air Soil Pollut. 21, 105-114.

Christiansen, J.R., Vesterdal, L., Callesen, I., Elberling, B., Schmidt, I.K., Gundersen, P., 2010. Role of six European tree species and land-use legacy for nitrogen and water budgets in forests. Glob. Change Biol. 16, 2224-2240.

Clarholm, M., Skyllberg, U., 2013. Translocation of metals by trees and fungi regulates pH, soil organic matter turnover and nitrogen availability in acidic forest soils. Soil Biol. Biochem. 63, 142-153.

Clemente, R., Dickinson, N.M., Lepp, N.W., 2008. Mobility of metals and metalloids in a multi-element contaminated soil 20 years after cessation of the pollution source activity. Environ. Pollut. 155, 254-261.

Compton, J.E., Church, M.R., Larned, S.T., Hogsett, W.E., 2003. Nitrogen export from forested watersheds in the Oregon Coast Range: the role of N_2-fixing red alder. Ecosystems 6, 773-785.

Cooke, J.E.K., Weih, M., 2005. Nitrogen storage and seasonal nitrogen cycling in *Populus*: bridging molecular physiology and ecophysiology. New Phytol. 167, 19-30.

Cornelis, W., Khlosi, M., Hartmann, R., Van Meirvenne, M., De Vos, B., 2005. Comparison of unimodal analytical expressions for the soil-water retention curve. Soil Sci. Soc. Am. J. 69, 1902-1911.

Cornelissen, J.H.C., 1996. An experimental comparison of leaf decomposition rates in a wide range of temperate plant species and types. J. Ecol. 84, 573-582.

Cornwell, W.K., Cornelissen, J.H.C., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy, O., Hobbie, S.E., Hoorens, B., Kurokawa, H., Perez-Harguindeguy, N., Quested, H.M., Santiago, L.S., Wardle, D.A., Wright, I.J., Aerts, R., Allison, S.D., Van Bodegom, P., Brovkin, V., Chatain, A., Callaghan, T.V., Díaz, S., Garnier, E., Gurvich, D.E., Kazakou, E., Klein, J.A., Read, J., Reich, P.B., Soudzilovskaia, N.A., Vaieretti, M.V., Westoby, M., 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecol. Lett. 11, 1065-1071.

Cotrufo, M.F., Miller, M., Zeller, B., 2000. Litter decomposition. In: Schulze, E.D. (Ed) Carbon and nitrogen cycling in European forest ecosystems. Springer, Berlin, pp 276-296.

Coughtrey, P.J., Jones, C.H., Martin, M.H., Shales, S.W., 1979. Litter accumulation in woodlands contaminated by Pb, Zn, Cd and Cu. Oecologia 39, 51-60.

Coûteaux, M., Bottner, P., Berg, B., 1995. Litter decomposition, climate and litter quality. Tree 10, 63-66.

Cronan, C.S., Aiken, R.G., 1985. Chemistry and transport of soluble humic substances in forested watersheds of the Adirondack Park, New York. Geochim. Cosmochim. Ac. 49, 1697-1705.

Crow, T.R., Laidly, P.R., 1980. Alternative models for estimating woody plant biomass. Can. J. Forest Res. 10, 367-370.

Currie, W.S., Aber, J.D., McDowell, W.H., Boone, R.D., Magill, A.H., 1996. Vertical transport of dissolved organic C and N under long-term N amendments in pine and hardwood forests. Biogeochemistry 35, 471-505.

Degryse, F., Vlassak, V., Smolders, E., Merckx, R., 2007. Mobilization of Cd upon acidification of agricultural soils: column study and field modelling. Eur. J. Soil Sci. 58, 152-165.

De Marco, A., Arena, C., Giordano, M., Virzo De Santo, A., 2013. Impact of the invasive tree black locust on soil properties of Mediterranean stone pine-holm oak forests. Plant Soil 372, 473-486.

De Schrijver, A., De Frenne, P., Staelens, J., Verstraeten, G., Muys, B., Vesterdal, L., Wuyts, K., Van Nevel, L., Schelfhout, S., De Neve, S., Verheyen, K., 2012. Tree species traits cause divergence in soil acidification during four decades of postagricultural forest development. Glob. Change Biol. 18, 1127-1140.

De Schrijver, A., Geudens, G., Augusto, L., Staelens, J., Mertens, J., Wuyts, K., Gielis, L., Verheyen, K., 2007. The effect of forest type on throughfall deposition and seepage flux: a review. Oecologia 153, 663-674.

De Schrijver, A., Mertens, J., Geudens, G., Staelens, J., Campforts, E., Luyssaert, S., De Temmerman, L., De Keersmaeker, L., De Neve, S., Verheyen, K., 2006. Acidification of forested podzols in North Belgium during the period 1950-2000. Sci. Total Environ. 361, 189-195.

De Schrijver, A., Nachtergale, L., Staelens, J., Luyssaert, S., De Keersmaeker, L., 2004. Comparison of throughfall and soil solution chemistry between a high-density Corsican pine stand and a naturally regenerated silver birch stand. Environ. Pollut. 131, 93-105.

De Schrijver, A., Staelens, J., Wuyts, K., Van Hoydonck, G., Janssen, N., Mertens, J., Gielis, L., Geudens, G., Augusto, L., Verheyen, K., 2008. Effect of vegetation type on throughfall deposition and seepage flux. Environ. Pollut. 153, 295-303.

Dickinson, N.M., 2000. Strategies for sustainable woodland on contaminated soils. Chemosphere 41, 259-263.

Dickinson, N.M., Pulford, I.D., 2005. Cadmium phytoextraction using short-rotation coppice *Salix*: the evidence trail. Environ. Int. 31, 609-613.

Dickinson, N.M., Watmough, S.A., Turner, A.P., 1996. Ecological impact of 100 years of metal processing at Prescot, northwest England. Env. Rev. 4, 8-24.

do Nascimento, C.W.A., Xing, B., 2006. Phytoextraction: a review on enhanced metal availability and plant accumulation. Sci. Agric. 63, 299-311.

Drohan, J.R., Sharpe, W.E., 1997. Long-term changes in forest soil acidity in Pennsylvania, USA. Water Air Soil Pollut. 95, 299-311.

Duursma, R.A., Medlyn, B.E., 2012. MAESPA: a model to study interactions between water limitation, environmental drivers and vegetation function at tree and stand levels, with an example application to [CO2] × drought interactions. Geosci. Model Dev. 5, 919-940.

Edmonds, R.L., 1980. Litter decomposition and nutrient release in Douglas fir, red alder, western hemlock and Pacific silver fir ecosystems in western Washington. Can. J. For. Res. 10, 327-337.

Edwards, C.A., 2004. Earthworm Ecology, 2nd ed. CRC Press, Boca Raton.

Emmer, I.M., 1995. Humus form development and succession of dwarf shrub vegetation in grass dominated primary *Pinus sylvestris* forests. Ann. Sci. For. 52, 561-571.

Emmer, I.M., Fanta, J., Kobus, A.T., Kooijman, A., Sevink, J., 1998. Reversing borealization as a means to restore biodiversity in Central-European mountain forests - an example from the Krkonoše Mountains, Czech Republic. Biodivers. Conserv. 7, 229-247.

Eriksson, J., Ledin, S., 1999. Changes in phytoavailability of cadmium in soil after long term *Salix* cropping. Water Air Soil Pollut. 114, 171-184.

Erisman, J.W., Draaijers, G., 2003. Deposition to forests in Europe: most important factors influencing dry deposition and models used for generalization. Environ. Pollut. 124, 379-388.

Ernst, W.H.O., 2005. Phytoextraction of mine wastes – options and impossibilities. Chemie der Erde 65 S1, 29-42.

European Union Risk Assessment Report, 2007. Cadmium oxide and cadmium metal, Part I – Environment, Volume 72.

Eviner, V.T., Chapin, F.S., 2003. Functional matrix: a conceptual framework for predicting multiple plant effects on ecosystem processes. Annu. Rev. Evol. Ecol. Syst. 34, 455-485.

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. PNAS 103, 626-631.

Finzi, A.C., Canham, C.D., van Breemen, N., 1998. Canopy tree-soil interactions within temperate forests: species effect on pH and cations. Ecol. Appl. 8, 447-454.

Fisher, R.F., Binkley, D., 2000. Ecology and management of forest soils, 3rd ed. John Wiley & Sons, New York.

Fischerová, Z., Tlustos, P., Száková J., Sichorová K., 2006. A comparison of phytoremediation capability of selected plant species for given trace elements. Environ. Pollut. 144, 93-100.

Fitz, W.J., Wenzel, W.W., Zhang, H., Nurmi, J., Kollensperger, G., Stipek, K., Fischerova, Z., Ma, L.Q., Stingeder, G., 2003. Rhizosphere characteristics of the arsenic hyperaccumulator *Pteris vittata* L. and monitoring of phytoremoval efficiency. Environ. Sci. Technol. 37, 5008-5014.

French, C.J., Dickinson, N.M., Putwain, P.D., 2006. Woody biomass phytoremediation of contaminated brownfield land. Environ. Pollut. 141, 387-395.

Fröberg, M., 2004. Processes controlling production and transport of dissolved organic carbon in forest soils. Dissertation, Swedish University of Agricultural Sciences, Uppsala.

Fröberg, M., Berggren Kleja, D., Bergkvist, B., Tipping, E., Mulder, J., 2005. Dissolved organic carbon leaching from a coniferous forest floor - a field manipulation experiment. Biogeochemistry 75, 271-287.

Fröberg, M., Hansson, K., Berggren Kleja, D., Alavi, G., 2011. Dissolved organic carbon and nitrogen leaching from Scots pine, Norway spruce and silver birch stands in southern Sweden. For. Ecol. Manag. 262, 1742-1747.

Gadd, G.M., Griffiths, A.J., 1978. Microorganisms and heavy metal toxicity. Microb. Ecol. 4, 303-317.

Garten, C.T., 1999. Modeling the potential role of a forest ecosystem in phytostabilization and phytoextraction of ^{90}Sr at a contaminated watershed. J. Environ. Radioact. 43, 305-323.

Gerrits, A.M.J., Pfister, L., Savenije, H.H.G., 2010. Spatial and temporal variability of canopy and forest floor interception in a beech forest. Hydrol. Process. 24, 3011-3025.

Gholz, H.L., Wedin, D.A., Smitherman, S.M., Harmon, M.E., Parton, W.J., 2000. Long-term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. Glob. Change Biol. 6, 751-765.

Gomes, H.I., 2012. Phytoremediation for bioenergy: challenges and opportunities. Environ. Technol. Rev. 1, 59-66.

Gosz, J.R., Likens, G.E., Bormann, F.H., 1973. Nutrient release from decomposing leaf and branch litter in the Hubbard Brook Forest, New Hampshire. Ecol. Monogr. 43, 173-191.

Greger, M., Landberg, T., 1999. Use of willow in phytoextraction. Int. J. Phytoremediat. 1, 115-123.

Grelle, A., Lundberg, A., Lindroth, A., Morén, A.S., Cienciala, E., 1997. Evaporation components of a boreal forest: variations during the growing season. J. Hydrol. 197, 70-87.

Hagen-Thorn, A., Callesen, I., Armolaitis, K., Nihlgård, B., 2004. The impact of six European tree species on the chemistry of mineral topsoil in forest plantations on former agricultural land. For. Ecol. Manag. 195, 373-384.

Haines, B.L., Waide, J.B., Todd, R.L., 1982. Soil solution nutrient concentrations sampled with tension and zero-tension lysimeters: report of discrepancies. Soil Sci. Soc. Am. J. 46, 658-661.

Hammer, D., Kayser, A., Keller, C., 2003. Phytoextraction of Cd and Zn with *Salix viminalis* in field trials. Soil Use Manag. 19, 187-192.

Hammer, D., Keller, C., 2002. Changes in the rhizosphere of heavy metal accumulating plants as evidenced by chemical extractants. J. Environ. Qual. 31, 1561-1569.

Hammer, D., Keller, C., 2003. Phytoextraction of Cd and Zn with *Thlaspi caerulescens* in field trials. Soil Use Manag. 19, 144-149.

Hamon, R.E, McLaughlin, M.J., 1999. Use of the hyperaccumulator *Thlaspi cearulescens* for bioavailable contaminant stripping. In: Wenzel, W.W., Adriano, D.C., Alloway, B., Doner, H., Keller, C., Lepp, N.W., Mench, M., Naidu, R., Pierzynski, G.M. (Eds) Extended Abstracts of the Fifth International Conference on the Biogeochemistry of Trace Elements (ICOBTE), Vienna, 908–909.

Hansen, K., Vesterdal, L., Schmidt, I.K., Gundersen, P., Sevel, L., Bastrup-Birk, A., Pederson, L.B., Bille-Hansen, J., 2009. Litterfall and nutrient return in five tree species in a common garden experiment. For. Ecol. Manag. 257, 2133-2144.

Harmon, M.E., Silver, W.L., Fasth, B., Chen, H., Burke, I.C., Parton, W.J., Hart, S.C., Currie, W.S., 2009. Long-term patterns of mass loss during the decomposition of leaf and fine root litter: an intersite comparison. Glob. Change Biol. 15, 1320-1338.

Hassinen, V., Vallinkoski, V-M., Issakainen, S., Tervahauta, A., Kärenlampi, S., Servomaa, K., 2009. Correlation of foliar *MT2b* expression with Cd and Zn concentrations in hybrid aspen (*Populus tremula x tremuloides*) grown in contaminated soil. Environ. Pollut. 157, 922-930.

Hättenschwiler, S., Coq, S., Barantal, S., Handa, I.T., 2011. Leaf traits and decomposition in tropical rainforests: revisiting some commonly held views and towards a new hypothesis. New Phytol. 189, 950-965.

Hättenschwiler, S., Gasser, P., 2005. Soil animals alter plant litter diversity effects on decomposition. PNAS 102, 1519-1524.

Hättenschwiler, S., Tiunov, A.V., Scheu, S., 2005. Biodiversity and litter decomposition in terrestrial ecosystems. Annu. Rev. Ecol. Evol. Syst. 36, 191-218.

Heal, O.W., Anderson, J.M., Swift, M.J., 1997. Plant litter quality and decomposition: a historical overview. In: Cadisch, G., Giller, K.E. (Eds) Driven by Nature: Plant Litter Quality and Decomposition. CAB International Wallingford, UK, pp 3-45.

Helling, C.S., Chesters, G., Corey, R.B., 1964. Contribution of organic matter and clay to soil cation exchange capacity as affected by the pH of the saturating solution. Soil Sci. Soc. Am. J. 28, 517-520.

Hinsinger, P. 2000. Bioavailability of trace elements as related to root-induced chemical changes in the rhizosphere. In: Gobran, G.R., Wenzel, W.W., Lombi, E. (Eds) Trace elements in the rhizosphere. CRC Press, Boca Raton, pp 25-41.

Herlitzius, H., 1983. Biological decomposition efficiency in different woodland soils. Oecologia 57, 78-97.

Hobbie, S.E., Ogdahl, M., Chorover, J., Chadwick, O.A., Oleksyn, J., Zytkowiak, R., Reich, P.B., 2007. Tree species effects on soil organic matter dynamics: the role of soil cation composition. Ecosystems 10, 999-1018.

Hobbie, S.E., Reich, P.B., Oleksyn, J., Ogdahl, M., Zytkowiak, R., Hale, C., Karolewski, P., 2006. Tree species effects on decomposition and forest floor dynamics in a common garden. Ecology 87, 2288-2297.

Hojjati, S.M., Hagen-Thorn, A., Lamersdorf, N.P., 2009. Canopy composition as a measure to identify patterns of nutrient input in a mixed European beech and Norway spruce forest in central Europe. Eur. J. For. Res. 128, 13-25.

Holm, P.E., Christensen, T.H., Tjell, J.C., McGrath, S.P., 1995. Speciation of cadmium and zinc with application to soil solutions. J. Environ. Qual. 24, 183-190.

Holmgren, G.G.S., Meyer, M.W., Chaney, R.L., Daniels, R.B., 1993. Cadmium, lead, zinc, copper, and nickel in agricultural soils of the United States of America. J. Environ. Qual. 22, 335-348.

Hommel, P., de Waal, R., Muys, B., den Ouden, J., Spek, T., 2007. Terug naar het lindewoud. KNNV Uitgeverij, Zeist.

Hotz, P., Buchet, J.P., Bernard, A., Lison, D., Lauwerys, R., 1999. Renal effects of low-level environmental cadmium exposure: 5-year follow-up of a subcohort from the Cadmibel study. Lancet 354, 1508-1513.

Hovmand, M.F., Bille-Hansen, J., 1999. Atmospheric input to Danish spruce forests and effects on soil acidification and forest growth based on 12 years measurements. Water Air Soil Pollut. 116, 75-88.

Howard, P.J.A., Howard, D.M., 1990. Titratable acids and bases in tree and shrub leaf litters. Forestry 63, 177-196.

Huang, J-H., Ilgen, G., Matzner, E., 2011. Fluxes and budgets of Cd, Zn, Cu, Cr and Ni in a remote forested catchment in Germany. Biogeochemistry 103, 59-70.

IARC, International Agency for Research on Cancer, 1993. Cadmium and cadmium compounds. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 58 – Beryllium, Cadmium, Mercury, and Exposures in the Glass Manufacturing Industry. Lyon, World Health Organization, 119-237.

IUSS Working Group WRB, 2006. World reference base for soil resources. World Soil Resources Reports No. 103. FAO, Rome.

Jabiol, B., Brêthes, A., Ponge, J.F., Toutain, F., Brun, J.J., 1995. L'humus sous toutes ses formes. École Nationale Du Génie Rural, des Eaux et des Forêts, Nancy.

Jacob, M., Weland, N., Platner, C., Schaefer, M., Leuschner, C., Thomas, F.M., 2009. Nutrient release from decomposing leaf litter of temperate deciduous forest trees along a gradient of increasing tree species diversity. Soil Biol. Biochem. 41, 2122-2130.

Jamaludheen, V., Kumar, B.M., 1999. Litter of multipurpose trees in Kerala, India: variations in the amount, quality, decay rates and release of nutrients. For. Ecol. Manag. 115, 1-11.

Järup, L., Berglund, M., Elinder, C.G., Nordberg, G., Vahter, M., 1998. Health effects of cadmium exposure - a review of the literature and a risk estimate. Scand. J. Work Environ. Health 24, suppl 1, 1-51.

Johansson, M.B., 1995. The chemical composition of needle and leaf litter from Scots pine, Norway spruce and white birch in Scandinavian forests. Forestry 68, 49-62.

Johnson, D.W., 1992. Base cation distribution and cycling. In: Johnson, D.W., Lindberg, S.E. (Eds) Atmospheric deposition and forest nutrient cycling. Ecological studies 91. Springer-Verlag, New York, pp 275-340.

Johnson, D., Hale, B., 2004. White birch (*Betula papyrifera* Marshall) foliar litter decomposition in relation to trace metal atmospheric inputs at metal-contaminated and uncontaminated sites near Sudbury, Ontario and Rouyn-Noranda, Quebec, Canada. Environ. Pollut. 127, 65-72.

Johnson, D.W., Lindberg, S.E., 1992. Atmospheric deposition and forest nutrient cycling. Ecological studies 91. Springer-Verlag, New York.

Johnston, A.E., Goulding, K.W.T., Poulton, P.R., 1986. Soil acidification during more than 100 years under permanent grassland and woodland at Rothamstead. Soil Use Manag. 2, 3-10.

Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers. Oikos 69, 373-386.

Jönsson, U., Rosengren, U., Thelin, G., Nihlgård, B., 2003. Acidification-induced chemical changes in coniferous forest soils in southern Sweden 1988–1999. Environ. Pollut. 123, 75–83.

Joris, I., Bronders, J., van der Grift, B., Seuntjens, P., 2014. Model-based scenario analysis of the impact of remediation measures on metal leaching from soils contaminated by historic smelter emissions. J. Environ. Qual. 43, 859-868.

Kacálek, D., Špulák, O., Dušek, D., Kuneš, I., Balcar, V., 2013. Species-specific properties of forest floor under mountain conditions. Austrian J. For. Sci. 130, 45-59.

Kaila, A., Asam, Z., Sarkkola, S., Xiao, L., Laurén, A., Vasander, H., Nieminen, M., 2012. Decomposition of harvest residue needles on peatlands drained for forestry – implications for nutrient and heavy metal dynamics. For. Ecol. Manag. 277, 141-149.

Kalbitz, K., Glaser, B., Bol, R., 2004. Clear-cutting of a Norway spruce stand: implications for controls on the dynamics of dissolved organic matter in the forest floor. Eur. J. Soil Sci. 55, 401-413.

Kalbitz, K., Meyer, A., Yang, R., Gerstberger, P., 2007. Response of dissolved organic matter in the forest floor to long-term manipulation of litter and throughfall inputs. Biogeochemistry 86, 301-318.

Kalbitz, K., Solinger, S., Park, J-H., Michalzik, B., Matzner, E., 2000. Controls on the dynamics of dissolved organic matter in soils: a review. Soil Sci. 165, 277-304.

Karlsson, G.P., Akselsson, C., Hellsten, S., Karlsson, P.E., 2011. Reduced European emissions of S and N - effects on air concentrations, deposition and soil water chemistry in Swedish forests. Environ. Pollut. 159, 3571-3582.

Keller, C., Hammer, D., 2004. Metal availability and soil toxicity after repeated croppings of *Thlaspi caerulescens* in metal contaminated soils. Environ. Pollut. 131, 243-254.

Keller, C., Hammer, D., Kayser, A., Richner, W., Brodbeck, M., Sennhauser, M., 2003. Root development and heavy metal phytoextraction efficiency: comparison of different plant species in the field. Plant Soil 249, 67-81.

Kelliher, F.M., Leuning, R., Schulze, E.D., 1993. Evaporation and canopy characteristics of coniferous forests and grasslands. Oecologia 95, 153-163.

Klang-Westin, E., Eriksson, J., 2003. Potential of *Salix* as phytoextractor for Cd on moderately contaminated soils. Plant Soil 249, 127-137.

Konova, M., 1966. Soil organic matter: its nature, its role in soil formation, and soil fertility. Pergamon, New York.

Kooijman, A.M., Emmer, I.M., Fanta, J., Sevink, J., 2000. Natural regeneration potential of the degraded Krkonoše forests. Land Degrad. Dev. 11, 459-473.

Koopmans, G.F., Römkens, P.F.A.M., Song, J., Temminghoff, E.J.M., Japenga, J., 2007. Predicting the phytoextraction duration to remediate heavy metal contaminated soils. Water Air Soil Pollut. 181, 355-371.

Köstner, B., 2001. Evaporation and transpiration from forests in Central Europe – relevance of patch-level studies for spatial scaling. Meteorol. Atmos. Phys. 76, 69-82.

Krämer, U., 2005. Phytoremediation: novel approaches to cleaning up polluted soils. Curr. Opin. Biotech. 16, 133-141.

Kutílek, M., Nielsen, D.R., 1994. Soil hydrology. Cremlingen-Destedt, Catena, 370 pp.

Landre, A.L., Watmough, S.A., Dillon, P.J., 2010. Metal pools, fluxes, and budgets in an acidified forested catchment on the Precambrian shield, Central Ontario, Canada. Water Air Soil Pollut. 209, 209-228.

Laskowski, R., Berg, B., 1993. Dynamics of some mineral nutrients and heavy metals in decomposing forest litter. Scand. J. For. Res. 8, 446-456.

Laskowski, R., Maryanski, M., Niklinska, M., 1994. Effect of heavy metals and mineral nutrients on forest litter respiration rate. Environ. Pollut. 84, 97-102.

Laskowski, R., Niklinska, M., Maryanski, M., 1995. The dynamics of chemical elements in forest litter. Ecology 76, 1393-1406.

Laureysens, I., Blust, R., De Temmerman, L., Lemmens, C., Ceulemans, R., 2004. Clonal variation in heavy metal accumulation and biomass production in a poplar coppice culture: I. Seasonal variation in leaf, wood and bark concentrations. Environ. Pollut. 131, 485-494.

Lauwerys, R., Amery, A., Bernard, A., Bruaux, P., Buchet, J.P., Claeys, F., De Plaen, P., Ducoffre, G., Fagard, R., Lijnen, P., Nick, L., Roels, H., Rondia, D., Saint-Remy, A., Sartor, F., Staessen, J.,

1990. Health effects of environmental exposure to cadmium: objectives, design and organization of the Cadmibel study: a cross-sectional morbidity study carried out in Belgium from 1985 to 1989. Environ. Health Persp. 87, 283-289.

Lepp, N.W., 1992. Uptake and accumulation of metals in bacteria and fungi. In: Adriano, D.C. (Ed) Biogeochemistry of Trace Metals – Advances in Trace Substances Research. Lewis Publishers, Boca Raton, pp 277-298.

Lepp, N.W., Dickinson, N.M., 2003. Natural bioremediation of metal-polluted soils - a case history from the U.K. In: Mench, M., Mocquut, B. (Eds) Risk assessment and sustainable land management using plants in trace element-contaminated soils. INRA, Bordeaux.

Linacre, N.A., Whiting, S.N., Angle, J.S., 2005. The impact of uncertainty on phytoremediation project costs. Int. J. Phytoremediat. 7, 259-269.

Linacre, N.A., Whiting, S.N., Baker, A.J.M., Angle, J.S., Ades, P.K., 2003. Transgenics and phytoremediation: the need for an integrated risk strategy assessment, management and communication strategy. Int. J. Phytoremediat. 5, 181-185.

Lindroos, A-J., Derome, J., Derome, K., Smolander, A., 2011. The effect of Scots pine, Norway spruce and silver birch on the chemical composition of stand throughfall and upper soil percolation water in northern Finland. Boreal Environ. Res. 16, 240-250.

Lomander, A., 2002. Organic matter turnover in forest and arable land. Dissertation, Swedish University of Agricultural Sciences, Uppsala.

Lomander, A., Johansson, M-B., 2001. Changes in concentrations of Cd, Zn, Mn, Cu and Pb in spruce (*Picea abies*) needle litter during decomposition. Water Air Soil Pollut. 132, 165-184.

Lorenz, K., Preston, C.M., Krumrei, S., Feger, K-H., 2004. Decomposition of needle/leaf litter from Scots pine, black cherry, common oak and European beech at a conurbation forest site. Eur. J. For. Res. 123, 177-188.

Lousier, J.D., Parkinson, D., 1976. Litter decomposition in a cool temperate deciduous forest. Can. J. Bot. 54, 419-436.

Ma, L.Q., Komar, K.M., Tu, C., Zhang, W., Cai, Y., Kennelley, E.D., 2001. A fern that accumulates arsenic. Nature 409, 579.

MacPherson, G., 1995. Home-grown energy from short-rotation coppice. Farming Press Books, Ipswich, UK.

Maddelein, D., Lust, N., 1992. Soil and forest floor characteristics of Scots pine stands on drift sands. Silva Gandavensis 57, 1-15.

Manzoni, S., Jackson, R.B., Trofymow, J.A., Porporato, A., 2008. The global stoichiometry of litter nitrogen mineralization. Science 321, 684-686.

Marchiol, L., Assolari, S., Sacco, P., Zerbi, G., 2004. Phytoextraction of heavy metals by canola (*Brassica napus*) and radish (*Raphanus sativus*) grown on multicontaminated soil. Environ. Pollut. 132, 21-27.

Marlow, J., Peart, J.R., 2014. Experimental reversal of soil acidification in a deciduous forest: implications for seedling performance and changes in dominance of shade-tolerant species. For. Ecol. Manag. 313, 63-68.

Marschner, H., 1995. Mineral Nutrition of Higher Plants, 2nd ed. Academic Press, London.

Martin, M.H., Coughtrey, P.J., 1987. Cycling and fate of heavy metals in a contaminated woodland ecosystem. In: Coughtrey, P.J., Martin, M.H., Unsworth, M.H. (Eds) Pollutant transport and fate in ecosystems, 6th ed. Blackwell, Oxford, pp 319.

Maxted, A.P., Black, C.R., West, H.M., Crout, N.M.J., McGrath, S.P., Young, S.D., 2007. Phytoextraction of cadmium and zinc by *Salix* from soil historically amended with sewage sludge. Plant Soil 290, 157-172.

Maxted, A.P., Young, S.D., Black, C.R., West, H.M., McGrath, S.P., 2003. Comparison of chemically-enhanced phytoextraction by arable crops and short rotation coppice with hyperaccumulator plants. 7th International Conference on the Biogeochemistry of Trace Elements (ICOBTE). Uppsala, Sweden.

Mayer, R., 1998. Soil acidification and cycling of metal elements: cause-effect relationships with regard to forestry practices and climatic changes. Agr. Ecosyst. Environ. 67, 145-152.

McBride, M., Sauvé, S., Hendershot, W., 1997. Solubility control of Cu, Zn, Cd and Pb in contaminated soils. Eur. J. Soil Sci. 48, 337-346.

McEnroe, N.A., Helmisaari, H.S., 2001. Decomposition of coniferous forest litter along a heavy metal pollution gradient, south-west Finland. Environ. Pollut. 113, 11-18.

McGrath, S.P., Lombi, E., Gray, C.W., Caille, N., Dunham, S.J., Zhao, F.J., 2006. Field evaluation of Cd and Zn phytoextraction potential by the hyperaccumulators *Thlaspi caerulescens* and *Arabidopsis halleri*. Environ. Pollut. 141, 115-125.

McGrath, S.P., Zhao, F.J., Lombi, E., 2001. Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils. Plant Soil 232, 207-214.

McLean, J.E., Bledsoe, B.E., 1992. Behavior of Metals in Soils. United States Environmental Protection Agency. EPA/540/S-92/018.

Meentemeyer, V., 1978. Macroclimate and lignin control of litter decomposition rates. Ecology 59, 465-472.

Meers, E., Lamsal, S., Vervaeke, P., Hopgood, M., Lust, N., Tack, F.M.G., 2005. Availability of heavy metals for uptake by *Salix viminalis* on a moderately contaminated dredged sediment disposal site. Environ. Pollut. 137, 354-364.

Meers, E., Tack, F.M.G., Verloo, M. G., 2008. Degradability of ethylenediaminedisuccinic acid (EDDS) in metal contaminated soils: implications for its use soil remediation. Chemosphere 70, 358-363.

Meers, E., Vandecasteele, B., Ruttens, A., Vangronsveld, J., Tack, F.M.G., 2007. Potential of five willow species (*Salix* spp.) for phytoextraction of heavy metals. Environ. Exp. Bot. 60, 57-68.

Meers, E., Van Slycken, S., Adriaensen, K., Ruttens, A., Vangronsveld, J., Du Laing, G., Witters, N., Thewys, T., Tack, F.M.G., 2010. The use of bio-energy crops (*Zea mays*) for 'phytoattenuation' of heavy metals on moderately contaminated soils: a field experiment. Chemosphere 78, 35-41.

Melillo, J.M., Aber, J.D., Muratore, J.F., 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63, 621-626.

Mertens, J., Luyssaert, S., Verbeeren, S., Vervaeke, P., Lust, N., 2001. Cd and Zn concentrations in small mammals and willow leaves on disposal facilities for dredged material. Environ. Pollut. 115, 17-22.

Mertens, J., Luyssaert, S., Verheyen, K., 2005. Use and abuse of trace metal concentrations in plant tissue for biomonitoring and phytoextraction. Environ. Pollut. 138, 1-4.

Mertens, J., Van Nevel, L., De Schrijver, A., Piesschaert, F., Oosterbaan, A., Tack, F.M.G., Verheyen, K., 2007. Tree species effect on the redistribution of soil metals. Environ. Pollut. 149, 173-181.

Mertens, J., Vervaeke, P., Meers, E., Tack, F.M.G., 2006. Seasonal changes of metals in willow (*Salix* sp.) stands for phytoremediation on dredged sediment. Environ. Sci. Technol. 40, 1962-1968.

Michalzik, B., Matzner, E., 1999. Dynamics of dissolved organic nitrogen and carbon in a Central European Norway spruce ecosystem. Eur. J. Soil Sci. 50, 579-590.

Michalzik, B., Kalbitz, K., Park, J-H., Solinger, S., Matzner, E., 2001. Fluxes and concentrations of dissolved organic carbon and nitrogen - a synthesis for temperate forests. Biogeochemistry 52, 173-205.

Miller, J.D., Duff, E.I., Hirst, D., Anderson, H.A., Bell, J.S., Henderson, D.J., 2001. Temporal changes in soil properties at an upland Scottish site between 1956 and 1997. Sci. Total Environ. 265, 15-26.

MIRA, 2010. Milieurapport Vlaanderen, Achtergronddocument 2010, Verspreiding van zware metalen. Peeters, B., Bierkens, J., Provoost, J., Den Hond, E., Van Volsem, S., Adriaenssens, E., Roekens, E., Bossuyt, M., Theuns, I., De Cooman, W., Eppinger, R., Frohnhoffs, A., D'hont, D., Geeraerts, C., Belpaire, C., Cardon, M., Ceenaeme, J., De Naeyer, F., Gommeren, E., Van Dyck, E., Vlaamse Milieumaatschappij, www.milieurapport.be

Mohr, D., Topp, W., 2005. Hazel improves soil quality of sloping oak stands in a German low mountain range. Ann. For. Sci. 62, 23-29.

Moran, M.D., 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. Oikos 100, 403-405.

Moro, M.J., Domingo, F., 2000. Litter decomposition in four woody species in a Mediterranean climate: weight loss, N and P dynamics. Ann. Bot. 86, 1065-1071.

Nawrot, T., Plusquin, M., Hogervorst, J., Roels, H.A., Celis, H., Thijs, L., Vangronsveld, J., Van Hecke, E., Staessen, J.A., 2006. Environmental exposure to cadmium and risk of cancer: a prospective population-based study. Lancet Oncol. 7, 119-126.

Nieminen, T., 2011. Soil solution collection and analysis. Manual Part XI, 30 pp. In: ICP Forests, Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests. UNECE ICP Forests Programme Co-ordinating Centre, Hamburg.

Nilsson, S.I., Miller, H.G., Miller, J.D., 1982. Forest growth as a possible cause of soil and water acidification - an examination of the concepts. Oikos 39, 40-49.

Nisbet, T., 2005. Water Use by Trees – information note. Forestry Commission, UK.

Nordén, U., 1994. Influence of tree species on acidification and mineral pools in deciduous forest soils of South Sweden. Water Air Soil Pollut. 76, 363-381.

Nosetto, M.D., Jobbágy, E.G., Paruelo, J.M., 2005. Land-use change and water losses: the case of grassland afforestation across a soil textural gradient in central Argentina. Glob. Change Biol. 11, 1101-1117.

Nowack, B., Schulin, R., Robinson, B.H., 2006. Critical assessment of chelant-enhanced metal phytoextraction. Environ. Sci. Technol. 40, 5225-5232.

Nye, P.H., 1981. Changes of pH across the rhizosphere induced by roots. Plant Soil 61, 7-26.

Olson, J.S., 1963. Energy storage and the balance of producers and decomposers in ecological systems. Ecology 44, 322-331.

Ovington, J.D., 1954. Studies of the development of woodland conditions under different trees: II. the forest floor. J. Ecol. 42, 71-80.

Ovington, J.D., 1958. Studies of the development of woodland conditions under different trees: VII. soil calcium and magnesium. J. Ecol. 46, 391-405.

Parton, W., Silver, W.L., Burke, I.C., Grassens, L., Harmon, M.E., Currie, W.S., King, J.Y., Adair, E.C., Brandt, L.A., Hart, S.C., Fasth, B., 2007. Global-scale similarities in nitrogen release patterns during long-term decomposition. Science 315, 361-364.

Pausas, J.G., 1997. Litter fall and litter decomposition in *Pinus sylvestris* forests of the eastern Pyrenees. J. Veg. Sci. 8, 643-650.

Peer, W.A., Baxter, I.R., Richards, E.L., Freeman, J.L., Murphy, A.S., 2005. Phytoremediation and hyperaccumulator plants. In: Tamás, M.J., Martinoia, E. (Eds) Molecular Biology of Metal Homeostasis and Detoxification. Topics in Current Genetics, Vol 14. Springer, Berlin, pp 299-340.

Peeters, K., 2006. Actieplan cadmium. http://www.lne.be/themas/milieu-en-gezondheid/acties/cadmiumproblematiek/actieplan cadmium.pdf

Perronnet, K., Schwartz, C., Gérard, E., Morel, J.L., 2000. Availability of cadmium and zinc accumulated in the leaves of *Thlaspi caerulescens* incorporated into the soil. Plant Soil 227, 257-263.

Pilon-Smits, E., 2005. Phytoremediation. Annu. Rev. Plant Biol. 56, 15-39.

Pilon-Smits, E.A.H., Freeman, J.L., 2006. Environmental cleanup using plants: biotechnological advances and ecological considerations. Front. Ecol. Environ. 4, 203-210.

Prescott, C.E., 2002. The influence of the forest canopy on nutrient cycling. Tree Physiol. 22, 1193-1200.

Prescott, C.E., 2005. Do rates of litter decomposition tell us anything we really need to know? For. Ecol. Manag. 220, 66-74.

Prescott, C.E., 2010. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils. Biogeochemistry 101, 133-149.

Prescott, C.E., Taylor, B.R., Parsons, W.F.J., Durall, D.M., Parkinson, D., 1993. Nutrient release from decomposing litter in Rocky Mountain coniferous forests: influence of nutrient availability. Can. J. For. Res. 23, 1576-1586.

Prescott, C.E., Vesterdal, L., Preston, C.M., Simard, S.W., 2004. Influence of initial chemistry on decomposition of foliar litter in contrasting forest types in British Columbia. Can. J. For. Res. 34, 1714-1729.

Price, A.G., Dunham, K., Carleton, T., Band, L., 1997. Variability of water fluxes through the black spruce (*Picea mariana*) canopy and feather moss (*Pleurozium schreberi*) carpet in the boreal forest of Northern Manitoba. J. Hydrol. 196, 310-323.

Pulford, I.D., Dickinson, N.M., 2005. Phytoremediation technologies using trees. In: Prasad, M.N.V., Saiwan, K.S., Naidu, R. (Eds) Trace elements in the environment. CRC Press, New York, pp 375-395.

Pulford, I.D., Watson, C., 2003. Phytoremediation of heavy metal-contaminated land by trees – a review. Environ. Int. 29, 529-540.

Pulido Moncada, M., Penning, L.H., Timm, L.C., Gabriels, D., Cornelis, W., 2014. Visual examinations and soil physical and hydraulic properties for assessing soil structural quality of soils with contrasting textures and land uses. Soil Till. Res. 140, 20-28.

Putuhena, W.M., Cordery, I., 1996. Estimation of interception capacity of the forest floor. J. Hydrol. 180, 283-299.

Qualls, R.G., Haines, B.L., 1991. Geochemistry of dissolved organic nutrients in water percolating through a forest ecosystems. Soil Sci. Soc. Am. J. 55, 1112-1123.

Qualls, R.G., Haines, B.L., Swank, W.T., 1991. Fluxes of dissolved organic nutrients and humic substances in a deciduous forest. Ecology 72, 254-266.

R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/.

Ranger, J., Nys, C., 1994. The effect of spruce (*Picea abies* Karst) on soil development: an analytical and experimental approach. Eur. J. Soil Sci. 45, 193-204.

Reich, P.B., Oleksyn, J., Modrzynski, J., Mrozinski, P., Hobbie, S.E., Eissenstat, D.M., Chorover, J., Chadwick, O.A., Hale, C.M., Tjoelker, M.G., 2005. Linking litter calcium, earthworms and soil properties: a common garden test with 14 tree species. Ecol. Lett. 8, 811-818.

Reimann, C., Arnoldussen, A., Boyd, R., Finne, T.E., Koller, F., Nordgulen, O., Englmaier, P., 2007. Element contents in leaves of four plant species (birch, mountain ash, fern and spruce) along anthropogenic and geogenic concentration gradients. Sci. Total Environ. 377, 416-433.

Rhoades, C., Oskarsson, H., Binkley, D., Stottlemyer, B., 2001. Alder (*Alnus crispa*) effects on soils in ecosystems of the Agashashok River valley, northwest Alaska. Ecoscience 8, 89-95.

Robinson, B.H., Brooks, R.R., Gregg, P.E.H., Kirkham, J.H., 1999. The nickel phytoextraction potential of some ultramafic soils as determined by sequential extraction. Geoderma 87, 293-304.

Robinson, B.H., Fernández, J-E., Madejón, P., Marañón, T., Murillo, J.M., Green, S., Clothier, B., 2003. Phytoextraction: an assessment of biogeochemical and economic viability. Plant Soil 249, 117-125.

Robinson, B.H., Leblanc, M., Petit, D., Brooks, R.R., Kirkham, J.H., Gregg, P.E.H., 1998. The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils. Plant Soil 203, 47-56.

Robinson, B.H., Mills, T.M., Petit, D., Fung, L.E., Green, S.R., Clothier, B.E., 2000. Natural and induced cadmium accumulation in poplar and willow: implications for phytoremediation. Plant Soil 227, 301-306.

Robinson, B.H., Schulin, R., Nowack, B., Roulier, S., Menon, M., Clothier, B., Green, S., Mills, T., 2006. Phytoremediation for the management of metal flux in contaminated sites. For. Snow Landsc. Res. 80, 221-234.

Rock, S.A., 1997. Land Remediation & Pollution Control. In: Freeman, H. (Ed) The Standard Handbook of Hazardous Waste Treatment and Disposal, 2nd ed. McGraw Hill, New York.

Römkens, P., de Vries, W., 1995. Acidification and metal mobilization: effects of land use changes on Cd mobility. Stud. Environ. Sci. 64, 367-380.

Römkens, P., Salomons, W., 1998. Cd, Cu and Zn solubility in arable and forest soils: consequences of land use changes for metal mobility and risk assessment. Soil Sci. 163, 859-871.

Ross, S.M., 1994. Toxic Metals in Soil-Plant Systems, 1st ed. John Wiley & Sons, New York.

Rosselli, W., Keller, C., Boschi, K., 2003. Phytoextraction capacity of trees growing on a metal contaminated soil. Plant Soil 256, 265-272.

Rovira, P., Rovira, R., 2010. Fitting litter decomposition datasets to mathematical curves: towards a generalised exponential approach. Geoderma 155, 329-343.

Salt, D.E., Smith, R.D., Raskin, I., 1998. Phytoremediation. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49, 643-668.

Sartor, F., Rondia, D., Claeys, F., Staessen, J., Lauwerys, R., Bernard, A., Buchet, J.P., Roels, H., Bruaux, P., Ducoffre, G., Lijnen, P., Thijs, L., Amery, A., 1992. Impact of environmental cadmium pollution on cadmium exposure and body burden. Arch. Environ. Health 47, 347-353.

Sas-Nowosielska, A., Kucharski, R., Malkowski, E., Pogrzeba, M., Kuperberg, J.M., Krynski, K., 2004. Phytoextraction crop disposal – an unsolved problem. Environ. Pollut. 128, 373–379.

Sauvé, S., Hendershot, W., Allen, H.E., 2000a. Solid-solution partitioning of metals in contaminated soils: dependence on pH, total metal burden, and organic matter. Environ. Sci. Technol. 34, 1125-1131.

Sauvé, S., Norvell, W.A., McBride, M., Hendershot, W., 2000b. Speciation and complexation of cadmium in extracted soil solutions. Environ. Sci. Technol. 34, 291-296.

Scheid, S., Günthardt-Goerg, M.S., Schulin, R., Nowack, B., 2009. Accumulation and solubility of metals during leaf litter decomposition in non-polluted and polluted soil. Eur. J. Soil Sci. 60, 613-621.

Schmidt, U., 2003. Enhancing phytoextraction: the effect of chemical soil manipulation on mobility, plant accumulation, and leaching of heavy metals. J. Environ. Qual. 32, 1939-1954.

Seuntjens, P., 2002. Field-scale cadmium transport in a heterogeneous layered soil. Water Air Soil Pollut. 140, 401-423.

Sevel, L., Hansen, H.C.B., Raulund-Rasmussen, K., 2009. Mass balance of cadmium in two contrasting oak forest ecosystems. J. Environ. Qual. 38, 93-102.

Šimůnek, J., Huang, K., Van Genuchten, M.T., 1998, 'The HYDRUS Code for Simulating the One-Dimensional Movement of Water, Heat and Multiple Solutes in Variably Saturated Media', Research Report, No. 144, U.S. Salinity Laboratory, USDA, Riverside, California. 142 pp.

Smith, V.C., Bradford, M.A., 2003. Litter quality impacts on grassland litter decomposition are differently dependent on soil fauna across time. Appl. Soil Ecol. 24, 197-203.

Smith, R.A.H., Bradshaw, A.D., 1972. Stabilization of toxic mine wastes by the use of tolerant plant populations. Trans. Inst. Min. Metall. Sect A. 81, 230-237.

Solinger, S., Kalbitz, K., Matzner, E., 2001. Controls on the dynamics of dissolved organic carbon and nitrogen in a Central European deciduous forest. Biogeochemistry 55, 327-349.

Staaf, H., 1980. Release of plant nutrients from decomposing leaf litter in a South Swedish beech forest. Ecography 3, 129-136.

Staaf, H., 1987. Foliage litter turnover and earthworm populations in three beech forests of contrasting soil and vegetation types. Oecologia 72, 58-64.

Staaf, H., Berg, B., 1982. Accumulation and release of plant nutrients in decomposing Scots pine needle litter - long-term decomposition in a Scots pine forest II. Can. J. Bot. 60, 1561-1568.

Staessen, J., Amery, A., Bernard, A., Bruaux, P., Buchet, J.P., Claeys, F., De Plaen, P., Ducoffre, G., Fagard, R., Lauwerys, R.R., Lijnen, P., Nick, L., Saint Remy, A., Roels, H., Rondia, D., Sartor, F., Thijs, L., 1991. Effects of exposure to cadmium on calcium metabolism: a population study. Br. J. Ind. Med. 48, 710-714.

Staessen, J.A., Lauwerys, R.R., Ide, G., Roels, H.A., Vyncke, G., Amery, A., 1994. Renal function and historical environmental cadmium pollution from zinc smelters. Lancet 343, 1523-1527.

Staessen, J.A., Roels, H.A., Emelianov, D., Kuznetsova, T., Thijs, L., Vangronsveld, J., Fagard, R., 1999. Environmental exposure to cadmium, forearm bone density, and risk of fractures: prospective population study. Lancet 353, 1140-1144.

Staessen, J., Roels, H., Vangronsveld, J., Clijsters, H., De Schrijver, K., De Temmerman, L., Dondeyne, F., Van Hulle, S., Wildemeersch, D., Wilms, L., 1995. Preventiemaatregelen voor bodemverontreiniging met cadmium. Tijdschr. Voor Geneeskunde 51, 1387-1395.

Starfinger, U., Kowarik, I., Rode, M., Schepker, H., 2003. From desirable ornamental plant to pest to accepted addition to the flora? – the perception of an alien tree species through the centuries. Biol. Invasions 5, 323-335.

Stevenson, F.J., 1982. Humus chemistry: genesis, composition, reactions. John Wiley & Sons, New York.

Strobel, B.W., Borggaard, O.K., Hansen, H.C.B., Andersen, M.K., Raulund-Rasmussen, K., 2005. Dissolved organic carbon and decreasing pH mobilize cadmium and copper in soil. Eur. J. Soil Sci. 56, 189-196.

Strobel, B.W., Hansen, H.C.B., Borggaard, O.K., Andersen, M.K., Raulund-Rasmussen, K., 2001a. Composition and reactivity of DOC in forest floor soil solutions in relation to tree species and soil type. Biogeochemistry 56, 1-26.

Strobel, B.W., Hansen, H.C.B., Borggaard, O.K., Andersen, M.K., Raulund-Rasmussen, K., 2001b. Cadmium and copper release kinetics in relation to afforestation of cultivated soil. Geochim. Cosmochim. Ac. 65, 1233-1242.

Swift, M.J., Heal, O.W., Anderson, J.M., 1979. Decomposition in Terrestrial Ecosystems. University of California Press, Berkeley.

Tateno, R., Tokuchi, N., Yamanaka, N., Du, S., Otsuki, K., Shimamura, T., Xue, Z., Wang, S., Hou, Q., 2007. Comparison of litterfall production and leaf litter decomposition between an exotic black locust plantation and an indigenous oak forest near Yan'an on the Loess Plateau, China. For. Ecol. Manag. 241, 84-90.

Temminghoff, E.J.M., Van der Zee, S.E.A.T.M., De Haan, F.A.M., 1995. Speciation and calcium competition effects on cadmium sorption by sandy soil at various pHs. Eur. J. Soil Sci. 46, 649-655.

Tipping, E., Hurley, M.A., 1992. A unifying model of cation binding by humic substances. Geochim. Cosmochim. Ac. 56, 3627-3641.

Trum, F., Titeux, H., Ranger, J., Delvaux, B., 2011. Influence of tree species on carbon and nitrogen transformation patterns in forest floor profiles. Ann. For. Sci. 68, 837-847.

Tyler, G., 1976. Heavy metal pollution, phosphatase activity, and mineralization of organic phosphorus in forest soils. Soil Biol. Biochem. 8, 327-332.

Tyler, G., 2005. Changes in the concentrations of major, minor and rare-earth elements during leaf senescence and decomposition in a *Fagus sylvatica* forest. For. Ecol. Manag. 206, 167-177.

Ukonmaanaho, L., Starr, M., Mannio, J., Ruoho-Airola, T., 2001. Heavy metal budgets for two headwater forested catchments in background areas of Finland. Environ. Pollut. 114, 63-75.

Unterbrunner, R., Puschenreiter, M., Sommer, P., Wieshammer, G., Tlustoš, P., Zupan, M., Wenzel, W.W., 2007. Heavy metal accumulation in trees growing on contaminated sites in Central Europe. Environ. Pollut. 148, 107-114.

Vandecasteele, B., Lauriks, R., De Vos, B., Tack, F.M.G., 2003. Cd and Zn concentration in hybrid poplar foliage and leaf beetles grown on polluted sediment-derived soils. Environ. Monit. Assess. 89, 263-283.

Vandenbruwane, J., 2008. Dynamics of dissolved organic matter in forest ecosystems in Flanders. Dissertation, Ghent University.

Vandermeer, J., 1989. The Ecology of Intercropping. Cambridge University Press, New York.

Van der Grift, B., Griffioen, J., 2008. Modelling assessment of regional groundwater contamination due to historic smelter emissions of heavy metals. J. Contam. Hydrol. 96, 48-68.

Van der Salm, C., Reinds, G.J., de Vries, W., 2004. Assessment of the water balance of European forests: a model study. Water Air Soil Pollut. Focus 4, 175-190.

Vangronsveld, J., Herzig, R., Weyens, N., Boulet, J., Adriaensen, K., Ruttens, A., Thewys, T., Vassilev, A., Meers, E., Nehnevajova, E., van der Lelie, D., Mench, M., 2009. Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ. Sci. Pollut. Res. 16, 765-794.

Vangronsveld, J., Sterckx, J., Van Assche, F., Clijsters, H., 1995a. Rehabilitation studies on an old non-ferrous waste dumping ground: effects of revegetation and metal immobilization by beringite. J. Geochem. Explor. 52, 221-229.

Vangronsveld, J., Van Assche, F., Clijsters, H., 1995b. Reclamation of a bare industrial area contaminated by non-ferrous metals: in situ metal immobilization and revegetation. Environ. Pollut. 87, 51-59.

Vanguelova, E.I., Benham, S., Pitman, R., Moffat, A.J., Broadmeadow, M., Nisbet, T., Durrant, D., Barsoum, N., Wilkinson, M., Bochereau, F., Hutchings, T., Broadmeadow, S., Crow, P., Taylor, P., Durrant Houston, T., 2010. Chemical fluxes in time through forest ecosystems in the UK – soil response to pollution recovery. Environ. Pollut. 158, 1857-1869.

Van Miegroet, H., Cole, D.W., 1984. The impact of nitrification on soil acidification and cation leaching in a red alder ecosystem. J. Environ. Qual. 13, 586-590.

Van Miegroet, H., Cole, D.W., 1985. Acidification sources in red alder and Douglas-fir soils - importance of nitrification. Soil Sci. Soc. Am. J. 49, 1274-1279.

Van Miegroet, H., Cole, D.W., Foster, N.W., 1992. Nitrogen distribution and cycling. In: Johnson, D.W., Lindberg, S.E. (Eds) Atmospheric deposition and forest nutrient cycling. Ecological studies 91. Springer-Verlag, New York, pp 178-196.

Van Slycken, S., 2011. The use of energy crops on metal contaminated agricultural soils in the Campine region. Dissertation, Ghent University.

Verstraeten, W.W., Muys, B., Feyen, J., Veroustraete, F., Minnaert, M., Meiresonne, L., De Schrijver, A., 2005. Comparative analysis of the actual evapotranspiration of Flemish forest and cropland, using the soil water balance model WAVE. Hydrol. Earth Syst. Sc.9, 225-241.

Verstraeten, A., Neirynck, J., Genouw, G., Cools, N., Roskams, P., Hens, M., 2012. Impact of declining atmospheric deposition on forest soil solution chemistry in Flanders, Belgium. Atmos. Environ. 62, 50-63.

Vervaeke, P., Luyssaert, S., Mertens, J., Meers, E., Tack, F.M.G., Lust, N., 2003. Phytoremediation prospects of willow stands on contaminated sediment: a field trial. Environ. Pollut. 126, 275–282.

Vervaeke, P., 2004. Phytoremediation of land disposed contaminated dredged sediments: fate of heavy metals. Dissertation, Ghent University.

Vervaeke, P., Tack, F.M.G., Lust, N., Verloo, M., 2004. Short- and longer-term effects of the willow root system on metal extractability in contaminated dredged sediment. J. Environ. Qual. 33, 976-983.

Vesterdal, L., Ritter, E., Gundersen, P., 2002. Change in soil organic carbon following afforestation of former arable land. For. Ecol. Manag. 169, 137-147.

Vesterdal, L., Schmidt, I.K., Callesen, I., Nilsson, L.O., Gundersen, P., 2008. Carbon and nitrogen in forest floor and mineral soil under six common European tree species. For. Ecol. Manag. 255, 35-48.

Virzo De Santo, A., Fierro, A., Berg, B., Rutigliano, F.A., De Marco, A., 2002. Heavy metals and litter decomposition in coniferous forests. Dev. Soil Sci. 28A, 63-78.

Vitousek, P.M., Turner, D.R., Parton, W.J., Sanford, R.L., 1994. Litter decomposition on the Mauna-Loa environmental matrix, Hawaii – patterns, mechanisms, and models. Ecology 75, 418-429.

Vlarebo, 2008. Besluit van de Vlaamse Regering houdende vaststelling van het Vlaams reglement betreffende de bodemsanering en de bodembescherming.

Voegelin, A., Barmettler, K., Kretzschmar, R., 2003. Heavy metal release from contaminated soils: comparison of column leaching and batch extraction results. J. Environ. Qual. 32, 865-875.

Waterinckx, M., Roelandt, B., 2001. De bosinventaris van het Vlaamse Gewest. Ministerie van de Vlaamse Gemeenschap. Afdeling Bos & Groen, Brussel, Belgie.

Watmough, S.A., Dillon, P.J., Epova, E.N., 2005. Metal partitioning and uptake in central Ontario forests. Environ. Pollut. 134, 493-502.

Wedderburn, M.E., Carter, J., 1999. Litter decomposition by four functional tree types for use in silvopastoral systems. Soil Biol. Biochem. 31, 455-461.

Weihermüller, L., Siemens, J., Deurer, M., Knoblauch, S., Rupp, H., Göttlein, A., Pütz, T., 2007. In situ soil water extraction: a review. J. Environ. Qual. 36, 1735-1748.

Weng, L., Temminghoff, E.J.M., Lofts, S., Tipping, E., Van Riemsdijk, W.H., 2002. Complexation with dissolved organic matter and solubility control of heavy metals in a sandy soil. Environ. Sci. Technol. 36, 4804-4810.

Weng, L., Temminghoff, E.J.M., Van Riemsdijk, W.H., 2001a. Contribution of individual sorbents to the control of heavy metal activity in sandy soil. Environ. Sci. Technol. 35, 4436-4443.

Weng, L., Temminghoff, E.J.M., Van Riemsdijk, W.H., 2001b. Determination of the free ion concentration of trace metals in soil solution using a soil column Donnan membrane technique. Eur. J. Soil Sci. 52, 629-637.

Whiting, S.N., Leake, J.R., McGrath, S.P., Baker, A.J.M., 2001. Assessment of Zn mobilization in the rhizosphere of *Thlaspi caerulescens* by bioassay with non-accumulator plants and soil solution. Plant Soil 237, 147-156.

WHO, World Health Organization, 2000. Air Quality Guidelines for Europe, 2nd ed. WHO Regional Publications, European Series, No. 91.

Wickings, K., Grandy, A.S., Reed, S.C., Cleveland, C.C., 2012. The origin of litter chemical complexity during decomposition. Ecol. Lett. 15, 1180-1188.

Wiklander, L., Andersson, A., 1972. The replacing efficiency of hydrogen ion in relation to base saturation and pH. Geoderma 7, 159-165.

Wilkens, B.J., Loch, J.P.G., 1997. Accumulation of cadmium and zinc from diffuse immission on acid sandy soils, as a function of soil composition. Water Air Soil Pollut. 96, 1-16.

Wolters, V., Schaefer, M., 1993. Effects of burrowing by the earthworm *Aporrectodea caliginosa* (Savigny) on beech litter decomposition in an agricultural and in a forest soil. Geoderma 56, 627-632.

Wuyts, K., De Schrijver, A., Staelens, J., Gielis, L., Vandenbruwane, J., Verheyen, K., 2008. Comparison of forest edge effects on throughfall deposition in different forest types. Environ. Pollut. 156, 854-861.

Zhang, H., Davison, W., Knight, B., McGrath, S., 1998. In situ measurements of solution concentrations and fluxes of trace metals in soils using DGT. Environ. Sci. Technol. 32, 704-710.

Zhang, L., Dawes, W.R., Walker, G.R., 2001. Response of mean annual evapotranspiration to vegetation changes at catchment scale. Water Resour. Res. 37, 701-708.

Zhang, D., Hui, D., Luo, Y., Zhou, G., 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. J. Plant Ecol. 1, 85-93.

Zianis, D., Mencuccini, M., 2004. On simplifying allometric analyses of forest biomass. For. Ecol. Manag. 187, 311-332.

Zirlewagen, D., Von Wilpert, K., 2001. Modeling water and ion fluxes in a highly structured, mixed-species stand. For. Ecol. Manag. 143, 27-37.

Curriculum vitae

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Education

2001-2004	MSc Bioscience Engineering: Land and Forest Management Ghent University
1998-2001	BSc Bioscience Engineering Ghent University
1992-1998	Secondary school (Latin-Mathematics) Koninklijk Atheneum Voskenslaan, Gent

Professional experience

June 2014 – present

Teaching assistant at the Department of Forest and Water Management, Faculty of Bioscience Engineering, Ghent University

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Junior assistant at Forest & Nature Lab, Department of Forest and Water Management, Faculty of Bioscience Engineering, Ghent University

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Scientific researcher at Forest & Nature Lab (Ghent University) on the project 'Validatie en optimalisatie bosvriendelijke houtexploitatie in Vlaanderen' financed by ANB

January 2006 – January 2007

Different projects at BOS+ (ngo)

September – December 2005

Scientific researcher at Forest & Nature Lab (Ghent University) on the project 'Bepaling en visualisatie van de kritische lasten voor zware metalen in Vlaanderen' financed by VMM

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Scientific researcher at Forest & Nature Lab (Ghent University) on the project 'Literatuurstudie en evaluatierapport van bufferfuncties van landschapsdijken' financed by Havenbedrijf Antwerpen

Scientific publications

Publications in international peer-reviewed journals

De Moor, S., Van Nevel, L., Michels, E., De Fraeye, M., Annicaert, B., Tack, F.M.G., Vangronsveld, J. & Meers, E. Limitations for practical implementation of phytoremediation with short rotation coppice - evidence from 6 years of field trials. Submitted to International Journal of Phytoremediation.

Van Nevel, L., Mertens, J., De Schrijver, A., De Neve, S. & Verheyen, K. (2014). Can shrub species with higher litter quality mitigate soil acidification in pine and oak forests on poor sandy soils? Forest Ecology and Management, 330, 38-45.

Van Nevel, L., Mertens, J., Demey, A., De Schrijver, A., De Neve, S., Tack, F.M.G. & Verheyen, K. (2014). Metal and nutrient dynamics in decomposing tree litter on a metal contaminated site. Environmental Pollution, 189, 54-62.

Van Nevel, L., Mertens, J., De Schrijver, A., Baeten, L., De Neve, S., Tack, F.M.G., Meers, E. & Verheyen, K. (2013). Forest floor leachate fluxes under six different tree species on a metal contaminated site. Science of the Total Environment, 447, 99-107.

Ampoorter, E., De Schrijver, A., Van Nevel, L., Hermy, M. & Verheyen, K. (2012). Impact of mechanized harvesting on compaction of sandy and clayey forest soils: results of a meta-analysis. Annals of Forest Science, 69, 533-542.

De Schrijver, A., De Frenne, P., Staelens, J., Verstraeten, G., Muys, B., Vesterdal, L., Wuyts, K., Van Nevel, L., Schelfhout, S., De Neve, S. & Verheyen, K. (2012). Tree species traits cause divergence in soil acidification during four decades of postagricultural forest development. Global Change Biology, 18, 1127-1140.

Van Nevel, L., Mertens, J., Staelens, J., De Schrijver, A., Tack, F., De Neve, S., Meers, E. & Verheyen, K. (2011). Elevated Cd and Zn uptake by aspen limits the phytostabilization potential compared to five other tree species. Ecological Engineering, 37, 1072-1080.

De Schrijver, A., De Frenne, P., Ampoorter, E., Van Nevel, L., Demey, A., Wuyts, K. & Verheyen, K. (2011). Cumulative nitrogen input drives species loss in terrestrial ecosystems. Global Ecology and Biogeography, 20, 803-8016.

Wuyts, K., De Schrijver, A., Staelens, J., Van Nevel, L., Adriaenssens, S. & Verheyen, K. (2011). Soil inorganic N leaching in edges of different forest types subject to high N deposition loads. Ecosystems, 14, 818-834.

Ampoorter, E., Van Nevel, L., De Vos, B., Hermy, M. & Verheyen, K. (2010). Assessing the effects of initial soil characteristics, machine mass and traffic intensity on forest soil compaction. Forest Ecology and Management, 260, 1664-1676.

Ampoorter, E., Van Nevel, L. & Verheyen, K. (2009). Soil damage after skidding: results of a Flemish field experiment. Berichte Freiburger Forstliche Forschung, 79, 65-70.

Van Nevel, L., Mertens, J., Oorts, K., Bogaert, G. & Verheyen, K. (2007). Phytoextraction of metals from soils: how far from practice? Environmental Pollution, 150, 34-40.

Mertens, J., Van Nevel, L., De Schrijver, A., Piesschaert, F., Oosterbaan, A., Tack, F.M.G. & Verheyen, K. (2007). Tree species effect on the redistribution of soil metals. Environmental Pollution, 149, 173-181.

Ampoorter, E., Van Nevel, L., Verheyen, K., Hermy, M. & De Vos, B. (2007). Effect of soil characteristics, machine weight and traffic intensity on soil compaction by forest harvesting. Communications in Agricultural and Applied Biological Sciences, 72 (1), 87-91.

Scientific reports

Verheyen K., De Schrijver A., Staelens J., Baeten L., De Frenne P., Adriaenssens S., Verstraeten G., Ampoorter E., Van Nevel L., Demey A., Wuyts K., Gruwez R. (2010). Pilootstudie naar kwantificering van de relaties tussen de achteruitgang van biodiversiteit en chronische overschrijding van kritische lasten. Studie in opdracht van het Instituut voor Natuur- en Bosonderzoek (Natuurrapportering).

Ampoorter, E., Van Nevel, L., De Vos, B., Goris, R. & Verheyen, K. (2008). Validatie en optimalisatie bosvriendelijke houtexploitatie in Vlaanderen, Eindrapport. In opdracht van Ministerie van de Vlaamse Gemeenschap – Agentschap voor Natuur en Bos, uitgevoerd door Universiteit Gent – Labo voor Bosbouw, Instituut voor Natuur- en Bosonderzoek, Inverde. 214 p.

Gobin, A., Van Nevel, L., Vanden Auweele, W., Willems, E., Verlinden, G. & Verheyen, K. (2006). Bepaling kritische lasten voor zware metalen. Studie uitgevoerd in opdracht van de Vlaamse Milieumaatschappij, MIRA, MIRA/2005/06, Labo voor Bosbouw (UGent) en Bodemkundige Dienst van België. 112 p.

Van Nevel, L. (2005). Evaluatierapport 'Bufferfuncties van beplante landschapsdijken'. Hoofdstuk in: Mertens, J. & Piesschaert, F. (2005) 'Onderzoeksproject Landschapsdijken. Risico's, ontwikkelingsmogelijkheden en beheer van dijken uit brak baggerslib', Instituut voor Natuurbehoud en Laboratorium voor Bosbouw (UGent). In opdracht van het Gemeentelijk Havenbedrijf Antwerpen. p. 215-254.

Chapters in books

De Schrijver, A., Wuyts, K., Van Nevel, L. & Mohren, F. (2010). Nutriëntenbeheer. In: Den Ouden, J., Muys, B., Mohren, F., Verheyen, K. (Eds.) Bosecologie en Bosbeheer. Acco, Leuven, pp. 403-415.

National publications without peer-review

Lotte Van Nevel, Darline Velghe, Lander Baeten & Kris Verheyen (2009). Exploiteren in eigen regie, waarom nie? Bosrevue 28, p. 1-7.

Rik De Vreese, Lore Provoost, Lotte Van Nevel, Katrien Laveren, Bert Geysels & Bert De Somviele (2008). Vademecum bosuitbreiding en bosverbinding door lokale besturen. Vereniging voor Bos in Vlaanderen, 93 p.

Rik De Vreese, Katrijn Gijsel, Lotte Van Nevel, Frank Strynck, Petra Detienne, Gudrun Van Langenhove en Patrick Huvenne (2006). Actiegerichte handleiding speelbossen - de realisatie van speelbossen stap voor stap, voor gemeentebesturen, -diensten, milieu- en jeugdraden. 47 p.

Rik De Vreese & Lotte Van Nevel (2006). Een speelbos realiseren, gemakkelijker dan je denkt! Bosrevue 18, p. 7-10.

Scientific activities

Participation in congresses, symposia or workshops

Participation with oral presentation

2-6 July 2012:	'EUROSOIL 2012' European Confederation of Soil Science Societies (ECSSS) Bari, Italy
23-27 May 2010:	'SETAC Europe' – 20th Annual Meeting Sevilla, Spain
22 Sept 2010:	'Phytomanagement van metaal-verontreinigde gronden in de Kempen' UGent, INBO & UHasselt Gent, Belgium
27-29 Oct 2009:	'CSME 2009' - Contaminated Site Management in Europe Redox Technologies, Inc. Gent, Belgium
29 June - 3 July 2009:	'BIOGEOMON 2009' - 6th International Symposium on Ecosystem Behavior The University of Helsinki, Finnish Forest Research Institute (METLA) & Finnish Environment Institute (SYKE) Helsinki, Finland
19 March 2009:	Symposium 'Starters in het Bosonderzoek' Brussel, Belgium

Participation with poster presentation

7-8 April 2008:	Annual Symposium 'Ecology of Industrial Pollution: Remediation, restoration and preservation' British Ecological Society (BES) Birmingham, UK
22 March 2007:	Symposium 'Starters in het Bosonderzoek' Brussel, Belgium

Participation without presentation

13-15 August 2014:	'SILVA network Annual Conference' Bern, Switzerland
9 June 2011:	'Risicobeheer en aanpak van metaalverontreinigde bodems' Provinciale Hogeschool Limburg, UHasselt & UGent Hasselt, Belgium
8-10 June 2010:	'SARCLE 2010' - Sustainable Approaches to Remediation of Contaminated Land in Europe Redox Technologies, Inc. Gent, Belgium
23 June 2008:	Symposium 'BeNeKempen' OVAM & AbdK Turnhout, Belgium
25 January 2007:	Seminarie 'BeNeKempen' OVAM, AbdK & VITO Mol, Belgium
17 October 2006:	Topical day 'Biogeochemical response of forest vegetation to chronic pollution: processes, dynamics and modelling' SCK-CEN Mol, Belgium

Supervision of MSc thesis students

2011-2012:	Celine Joos: Duurzame stabilisatie van metaalverontreinigingen door middel van een uitgekiende boomsoortenkeuze
2010-2011:	Wim Verbist:Boomsoorteneffecten op strooiselafbraak op een met zware metalen verontreinigd terrein
2007-2008:	Arne Vermeulen: 'Effecten van boomsoortenkeuze op de bovengrondse compartimentering van Cd en Zn na bebossing van verontreinigde landbouwgronden in de Kempen'
2007-2008:	Thomas de Wilde: 'Boomsoorteffecten op de Cd- en Zn-dynamiek in de bodem na bebossing van verontreinigde landbouwgronden in de Kempen'